Errata - April 1997
Report of the Great Lakes Anglers
Pilot Exposure Assessment Report (April 1995)

Please note the following errors and corrections to be made to the report as of April 1997.

P. 13, para. 2, line 11 - 'serum levels ...' should be 'plasma levels...'.

P. 13, para. 2, line 17 - 'No heptachlor was detected' should be 'Aldrin, endrin, heptachlor epoxide and α-chlordane were never detected (detection limits were 0.2 μg/L).'.

p. 13, para. 2, line 18 - '(i.e. 1 ug/L)' should be '(i.e. 0.1 μg/L)'.

P. 21 - see new Table 6-2 (with slight changes to # respondents)

p. 28, Table 6-5 - detection limits: DDE - 0.02 μg/L; total mercury in blood (1.1 μg/L), inorganic mercury in blood (1.1 μg/L). Detection limits for mercury in hair are actually limits of quantification. Only hair mercury values below the limit of quantification were reported by the laboratory.

P. 30, last para., line 2. Insert as 2nd sentence: 'Only values above the level of quantification (0.5 μg/g) were reported by the laboratory'.

P. 42, Table 7-2 - remove footnote.

P. 45, para 2, line 6 - 'The age range was 18 to >60 years ...' should be 'The age range was 23 to 69 years ..'.

P. 59, Figure 7-4 - The 4th bar (for Cornwall, 'Have a copy and follow advice') should be 74.1%, not 66.7%.

P. 60, Figure 7-5 - The figure is showing the percentage of sport fish eaters with 1991 household income greater than $45,000 (not $60,000).

P. 72, Table 7-22 - for PCB128, Cornwall, Great Lakes fish non-eaters (1st column) - range should be '<0.02' μg/L . For PCB128, Mississauga Great Lakes fish non-eaters (3rd column) - geometric standard deviation should be 1.00 and range should be <0.02 μg/L.

P. 76, Table 7-25 - For Mississauga Great Lakes fish non-eaters, the n should be 47, not 48. For Mississauga Great Lakes fish eaters, the n should be 42, not 43 (two samples have missing lipid values, so lipid adjustment could not be done).

P. 78, last para., line 2 - replace 'serum' with 'plasma'.

P. 78, last para, line 7 - replace 'The chlordanes were rarely detected and then only among sport fish eaters' with 'The chlordanes were rarely detected and then only among sport fish non-eaters.'

P. 80, Table 7-27 - For Trans-nonachlor, All Participants (last column) the range should be '0.03-0.98'. For α-chlordane, Cornwall Great Lakes fish eaters (2nd column) the range should be '<0.02'. Similary, for γ-chlordane, Cornwall Great Lakes fish eaters (2nd column) AND Mississauga Great Lakes fish non-eaters (3rd column) the ranges should be '<0.02'.

P. 81, Table 7-28 - For Mississauga Great Lakes fish non-eaters, the n should be 47, not 48. For Mississauga Great Lakes fish eaters, the n should be 42, not 43 (two samples have missing lipid values, so lipid adjustment could not be done).

P. 85, Table 7-31 - The title should be '.... in pooled plasma samples'.

P. 85, Table 7-31 - The 'Total PCB category' for Cornwall 'Eaters' >50 years should be 'higher', not 'lower'.

P. 85, Table 7-31 - the TEF for 234678-HxCDF (4th line in Dibenzofuran section) should be 0.1, not 0.01.

P. 85, Table 7-31 - the TEQ for Cornwall Eaters, <38 years, 'higher' total PCB category (column 2) should be 22.24, not 20.78.

P. 85, Table 7-31 - The coplanar PCB TEQ and Total TEQ for Cornwall Eaters, <38 years, 'lower' total PCB category, should both be N/A (not available).

P. 86-90 & Table 7-30 (p. 83) - See attached revisions for the blood mercury section. Since the report was written the laboratory discovered that the blood mercury detection limit was lower than originally reported (1.1 μg/L vs. 2.0 μg/L). This has resulted in an increase in the number of detectable measurements. The tables and text have been modified to reflect the new data provided by the laboratory.

p. 103, recommendation 11, line 2 - replace 'serum' with 'plasma'..

The data were coded and entered with 100% verification. Range and logic tests were conducted during data capture, and inconsistencies in responses were flagged. A data processing strategy and edit-code protocol was prepared by the contractor.

Table 6-2
Sampling frame and response rates for mail-out questionnaire

Community	Gender	Age[1] Category	# of license holders	# of license holders sampled	# of respondents (and percentages) to mail-out questionnaire
Cornwall	Female	18-39 yrs	1051	578	197 (34%)
		40-64 yrs	918	566	285 (50%)
	Male	18-39 yrs	3510	579	231 (40%)
		40-64 yrs	3043	565	270 (48%)
	TOTAL		**8522**	**2288**	**983 (43%)**
Mississauga	Female	18-39 yrs	2097	497	161 (32%)
		40-64 yrs	1194	496	215 (43%)
	Male	18-39 yrs	10497	499	161 (32%)
		40-64 yrs	7802	496	235 (47%)
	TOTAL		**21590**	**1989**	**772 (39%)**
OVERALL TOTALS				**4277**	**1755 (41%)**

[1] Age calculated as of Jan1/91.

Lancaster interviews

On the advice of the Eastern Ontario Public Health Unit, in the Lancaster area (Lancaster Township (Geocode 01 001), Lancaster Village (Geocode 01 003) and Charlottenburgh Township (Geocode 01 006)), three local health professionals conducted personal interviews with community residents. The Health Unit staff suggested that this might be a more effective way to reach high fish consumers in the area. The interview team worked in the community to identify people based on their fish consumption habits, not their fishing habits. This included people who may or may not have had a fishing licence in 1988, i.e. they may or may not have been in the sampling frame for the mail-out survey. A mail-out survey of 358 license holders also occurred in the Lancaster area (Geocodes 01 001, 01 003 and 01 006).

The interviewers were originally asked to seek out and interview non-native individuals who have eaten 26 or more meals of fresh-water fish in the past 12 months ('high' consumption group). The interviewers had difficulty in finding people consuming fish this frequently, so this criterion was dropped to 12 or more meals of fresh-water fish in the

7.7 *Metals*

Mercury

Total mercury levels in whole blood were above detection limit (1.1µg/L) in 87% of samples and ranged from <1.1 to 16.3 µg/L. The arithmetic mean level was 2.5 µg/L; the geometric mean was 2.0 µg/L. Inorganic mercury was detectable in only 58 of 226 samples (26%), and ranged from <1.1-3.15µg/L (six samples did not have sufficent volume for analysis). Methyl (organic) mercury levels were calculated by subtracting the inorganic mercury level from the total mercury level (when the total mercury level was above the detection limit and the inorganic mercury level was not missing). The methyl mercury levels in whole blood ranged from 0 to 15.8 µg/L; on average, methyl mercury accounted for 62% of the total mercury, although this proportion varied widely between individual samples.

Total mercury blood levels are reported by sport fish eating status and community in Table 7-32 (refer back to Table 7-5 for explanation of category). All total mercury blood levels were within the Health Canada 'normal acceptable range' of less than 20 µg/L.

Table 7-32
Total mercury in whole blood
(µg/L)

	Sport fish		
	"Non-eaters"	**"Eaters"**	**Both**
Cornwall			
n	28	113	141
n ≥ detection limit	19	102	121
arithmetic mean (standard deviation)[1]	1.4 (0.8)	2.8 (2.4)	2.5 (2.2)
geometric mean (standard deviation)[1]	1.1 (1.8)	2.2 (2.0)	1.9 (2.1)
range	<1.1-3.5	<1.1-16.3	<1.1-16.3
Mississauga			
n	28	63	91
n ≥ detection limit	24	57	81
arithmetic mean (standard deviation)[1]	2.3 (1.3)	2.7 (1.7)	2.6 (1.6)
geometric mean (standard deviation)[1]	1.9 (2.0)	2.2 (2.0)	2.1 (2.0
range	<1.1-5.4	<1.1-8.5	<1.1-8.5
Both			
n	56	176	232
n ≥ detection limit	43	159	202
arithmetic mean (standard deviation)[1]	1.8 (1.1)	2.8 (2.1)	2.5 (2.0)
geometric mean (standard deviation)[1]	1.5 (2.0)	2.2 (2.0)	2.0 (2.0)
range	<1.1-5.4	<1.1-16.3	<1.1-16.3

[1] Means and standard deviations were calculated after replacing non-detectable values with a value equal to ½ the detection limit (ie. 0.55µg/L).

[2] Wilcoxon rank sum test for differences in rank distributions between non-eaters and eaters was significant for males and females separately (both p=0.08) and combined (p=0.0016).

Mean levels were considerably below that found in the Québec Inuit survey (geo. mean = 15.0μg/L, Santé Québec 1994) and in a study of 179 commercial fishermen and their families along the Lower North Shore of the Gulf of St. Lawrence River (geo. mean = 5.4μg/L, Laliberté et al 1992). Levels were also lower than those reported from native populations prior to 1982 (Health & Welfare Canada 1979, 1984). The (arithmetic) mean mercury level for fish eaters in this study (2.8μg/L) is well below the reference value reported by Brune et al (1991) for fish consumers (8.4μg/L) (based on a review of 98 published studies). The mean total mercury level for non-eaters in this study (1.9μg/L) was similar to the reference value for non-eaters (2.0μg/L) (Brune et al 1991).

Statistical modelling was carried out on the natural logarithms of the blood (total) mercury levels; values below the detection limit were treated as ½ the detection limit. The following variables were included in a stepwise regression - sport fish consumption, ducks/geese consumption, smoking, age and gender. The resultant model is presented in Table 7-33. The model indicates blood mercury levels were 40% higher (p = 0.001) for sport fish consumers compared with non-consumers after adustment for age and gender. Ducks/geese consumption was not significant (p = 0.31) when added to this model.

Table 7-33
Model of ln (total mercury in blood)
Dependent variable: ln (Total mercury in blood - μg/L)
($r^2 = 0.12$)

Variable	Coefficient (βi)	Standard error (βi)	P-value Type I SS	P-Value Type III SS	$e^{\beta i}$
Intercept (INTERCEPT)	-0.157	0.235			0.86
Age (years) (AGE)	0.015	0.005	0.017	0.002	1.02
Gender (GENDER) (female = 1)	0.586	0.375	0.003	0.119	1.80
Age * Gender (AGE*GENDER)	-0.019	0.008	0.027	0.024	0.98
Sport fish consumption in last 12 months (1 = yes/0 = no) (SFN12MTH)	0.339	0.104	0.001	0.001	1.40

Hair mercury was analysed in 809 one centimeter samples from the 232 participants. Because of short hair, not all participants were able to provide the 1,2,3,5 and 7 cm segment samples. Total mercury levels were above the limit of quantification (0.5µg/g) in only 36% of the 809 samples and ranged from <0.5 to 8.4 µg/g (Table 7-34). Inorganic mercury was found above the limit of quantification (0.5µg/g) in only 17 of the 809 samples (2%) and ranged from <0.5-0.9µg/g. Again, methyl mercury levels were calculated by subtracting the inorganic mercury level from the total mercury level for each sample. Across all the samples, methyl mercury ranged from 0.1 to 7.8 µg/g. On average, methyl mercury accounted for 63% of total mercury.

Mercury concentrations in hair showed good correlation across segments (Table 7-35). The best correlation between blood and hair levels was in the proximal hair segments, in keeping with blood levels better reflecting more recent consumption. Only one participant had a hair mercury level of 8.4µg/g placing him in the "increasing risk" category specified by Health Canada (see appendix 7). (This participant was a fish eater; their blood mercury level was 11.3 µg/L).

Geometric mean hair mercury levels were considerably lower (0.28-0.45 µg/g) than levels found in Ontario Amerindians (males 2.7 µg/g, females 2.1 µg/g) (Richardson & Currie 1993) and in Quebec James Bay Cree (9.6 µg/g in 1988, Perrault 1992). Statistical modelling of the hair levels was not carried out due to the large number of non-detectable and missing values. However geometric mean mercury levels were higher in hair samples from Mississauga than in those from Cornwall for sport fish 'non-eaters'. Within the Cornwall samples, mean mercury levels were higher for sport fish eaters than non-eaters. However, in Mississauga mean mercury levels in hair were quite similar for sport fish eaters and non-eaters. These comparisons were not adjusted for any confounding variables.

Mercury levels in blood and hair were not highly correlated with organochlorine blood levels (refer back to Table 7-30). Indeed, mercury tends to be higher in fish from some inland lakes while organochlorines are higher in some Great Lakes locations.

Table 7-34
Total mercury in hair
(µg/g)

	Sport fish 'non-eaters' (n=56 participants)					Sport fish 'eaters' (n=176 participants)				
	Centimeter section					Centimeter section				
	1	2	3	5	7	1	2	3	5	7
Cornwall #≥0.5µg/g	3	3	4	1	1	54	52	41	16	8
#<0.5µg/g	25	25	22	16	10	59	59	49	26	14
number missing (no sample)	0	0	2	11	17	0	2	23	71	91
arith. mean	0.31(0.20)	0.30(0.14)	0.32(0.19)	0.28(0.11)	0.29(0.14)	0.65(0.92)	0.63(0.92)	0.59(0.79)	0.41(0.24)	0.40(0.22)
geo. mean	0.28(1.44)	0.28(1.38)	0.29(1.48)	0.27(1.28)	0.27(1.36)	0.45(2.10)	0.44(2.07)	0.43(2.01)	0.36(1.63)	0.36(1.64)
median	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
range	<0.5-1.2	<0.5-0.8	<0.5-0.9	<0.5-0.7	<0.5-0.7	<0.5-8	<0.5-8.4	<0.5-6.6	<0.5-1.3	<0.5-0.8
Mississauga #≥0.5µg/g	6	9	4	4	5	22	23	19	9	5
#<0.5µg/g	21	18	20	9	3	41	40	32	20	11
number missing (no sample)	1	1	4	15	20	0	0	12	34	47
arith. mean	0.45(0.44)	0.51(0.44)	0.38(0.31)	0.38(0.22)	0.48(0.23)	0.40(0.25)	0.43(0.30)	0.46(0.34)	0.41(0.27)	0.38(0.22)
geo. mean	0.35(1.90)	0.39(1.99)	0.31(1.69)	0.34(1.62)	0.43(1.64)	0.35(1.63)	0.37(1.72)	0.38(1.79)	0.35(1.72)	0.34(1.60)
median	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
range	<0.5-1.9	<0.5-1.6	<0.5-1.5	<0.5-0.9	<0.5-0.9	<0.5-1.5	<0.5-1.5	<0.5-1.7	<0.5-1.0	<0.5-1.0

---

[1] Means and standard deviations were calculated after replacing all non-detectable values with a value equal to ½ the detection limit (ie. 0.25µg/g).

Table 7-35
Spearman correlations between total mercury measurements in blood and hair[1]

		Total mercury in hair				
		cm 1	cm 2	cm 3	cm 5	cm 7
Total mercury in blood		0.44	0.42	0.39	0.39	0.19
Total mercury in hair	cm 1		0.83	0.68	0.64	0.62
	cm 2			0.78	0.76	0.62
	cm 3				0.80	0.56
	cm 5					0.71

[1] Correlations were calculated after replacing non-detectable values with a value equal to 1/2 the detection limit.

Report on the
Great Lakes Anglers Pilot Exposure Assessment Study

Great Lakes Health Effects Program
Health Canada

April 1995

Jill Kearney
Great Lakes Health Effects Program
Health Canada

Dr. Donald C. Cole
Environmental Health Program
McMaster University

Doug Haines
Great Lakes Health Effects Program
Health Canada

DRAFT

For more information or comments please contact:

Jill Kearney
Great Lakes Health Effects Program
Health Canada
Room 1141, Main Building
Tunney's Pasture
Ottawa, Ontario
K1A 0K9
(613) 957-8487

Table of Contents

1.0 Executive Summary 1
2.0 Acknowledgements 3
3.0 Introduction 5
4.0 Background Information 6
  4.1 Fishing activity in Ontario 6
  4.2 Fish consumption levels 7
  4.3 Concentrations of contaminants in Great Lakes fish 9
  4.4 Effect of fish preparation and cooking methods on fish contaminant levels 9
  4.5 Relationship between fish consumption and human blood contaminant levels 10
5.0 Study objectives and primary hypothesis 14
6.0 Methods 15
  6.1 Study sites 15
  6.2 Public consultation and information 17
  6.3 Population/sampling strategy 18
    6.3.1 Stage 1 - Methods used in mail-out questionnaire and Lancaster interviews 19
    6.3.2 Stage 1 - Creation of sampling frame for biological sampling 23
  6.4 Field methods 25
    6.4.1 Recruitment of participants including informed consent 25
    6.4.2 Questionnaire instrument and implementation 26
    6.4.3 Biological sampling 27
      6.4.3.1 Contaminant residue analysis 29
      6.4.3.2 Clinical chemistry and lipid profiles 31
      6.4.3.3 Other sub-clinical effect and exposure markers 31
  6.5 Stage 2 - Data management and analysis 34
  6.6 Dissemination of individual results 36
    6.6.1 Results reporting 36
    6.6.2 Feedback on results reporting from physicians 39
  6.7 Dissemination of study results 39
7.0 Results and discussion 40
  7.1 Participation rates 40
  7.2 Participant demographic characteristics 45
  7.3 Sport fish and wildlife consumption 46
  7.4 Other exposures and potential confounding variables 63
  7.5 Lipids 70
  7.6 Organochlorine compounds 71
  7.7 Metals (Mercury, lead, cadmium and urinary cotinine) 86
  7.8 Clinical chemistry - thyroid status and liver enzymes 100
  7.9 Subclinical effect markers - D-glucaric acid and urinary porphyrins 100
  7.10 Physician feedback on individual results dissemination 100
8.0 Conclusions and recommendations 102
9.0 References 107

Appendices

List of Tables

6-1 Framework for study implementation 18
6-2 Sample frame and response rates for mail-out questionnaire 21
6-3 Number of Lancaster interviews 22
6-4 'PCB index' distribution 24
6-5 Measurements in biological samples 28
6-6 Categories of fish consumers 34
7-1 Participation rates 41
7-2 Reasons for non-participation 42
7-3 Comparison of participants and non-participants 43
7-4 Number of participants and mean age (by sample and gender) 45
7-5 Sport fish consumption categories 46
7-6 Fish species eaten in last 12 months 47
7-7 Meal size by species 50
7-8 Meal size, meal frequency and total mass eaten in last 12 months 51
7-9 Mean sport fish consumption estimates in this and other studies 52
7-10 Cleaning, parts eaten and cooking summary measures 54
7-11 Historical fish consumption 55
7-12 Number of sport fish eaters reporting changes in amount eaten, species and location during last 10 years 56
7-13 Guide use and mean measures for cleaning, parts eaten and cooking 61
7-14 Percentage of participants consuming wildlife and waterfowl 63
7-15 Potential confounders 65
7-16 Percentages of participants reporting occupational and home chemical exposures 66
7-17 Number of women who had ever breastfed 68
7-18 Total number of months breastfeeding (lifetime) 68
7-19 Breastfeeding status (lifetime) and Great Lakes fish consumption 69
7-20 Serum lipids 71
7-21 Total serum lipids (g/L) 71
7-22 PCB plasma concentrations (wet weight µg/L) 72
7-23 Comparison of arithmetic mean levels of PCBs from this and other fisheater studies 73
7-24 Spearman rank correlations between PCB congeners (in plasma) 75
7-25 PCB plasma concentrations based on lipid weight (µg/kg) 76
7-26 Model of ln(AR1260) 77
7-27 Organochlorine pesticide concentrations in blood plasma (wet weight µg/L) 80
7-28 Organochlorine pesticide concentrations in blood plasma lipids (µg/kg lipid) 81
7-29 Comparison of arithmetic mean levels of organochlorines pesticides in blood from this and other fisheater studies 82
7-30 Spearman rank correlations between Aroclor 1260, organochlorine pesticides (in plasma), and mercury, lead, cadmium (in whole blood) 83
7-31 Dioxins, dibenzofurans and co-planar PCB levels in pooled serum samples 85
7-32 Total mercury in whole blood (µg/L) 86
7-33 Model of ln (total mercury in blood) 87
7-34 Total mercury in hair (µg/g) 89
7-35 Spearman correlations between total mercury measurements in blood and hair . . 90
7-36 Lead in whole blood (µg/dL) 91

7-37 Model of ln (lead in whole blood) 92
7-38 Cadmium in whole blood (µg/L) - sample status & percentages 94
7-39 Cadmium in whole blood (µg/L) by smoking status 95
7-40 Model of ln(cadmium in whole blood) 97
7-41 Urinary cotinine 98

List of Figures

6-1 Map of Cornwall and Mississauga study sites 16
6-2 Sampling process 20
6-3 Calculation of "PCB index" 24
6-4 Classes of biological markers 32
6-5 Materials sent to participants and physicians regarding individual results 38
7-1 Fishing locations 48
7-2 Fish cleaning, parts eaten and cooking method summary measures 54
7-3 Awareness and use of "Guide to Eating Ontario Sport Fish" 58
7-4 Awareness of guide and education level 59
7-5 Awareness of guide and income level 60
7-6 Smoking distribution 64
7-7 Cadmium in whole blood (µg/L) by smoking status 96
7-8 Urinary cotinine (µg/g creatinine) by smoking status 99

Appendices

1. Great Lakes Basin Anglers Survey instrument (mail-out/interview)
2. Consent form
3. Stage 2 questionnaire (interviewer-administered)
4. Methods used to calculate meal size
5. Fish preparation, parts eaten and cooking methods
6. Protocols for collection, processing, storage and shipping of blood, hair and urine samples
7. Dissemination of individual results - interpretation of biological sample results for participants and physicians
8. Dissemination of individual results - Examples of letters with preliminary clinical chemistry results
9. Dissemination of individual results - Examples of letters to participants and physicians for reporting of all results

1.0 EXECUTIVE SUMMARY

In the fall/winter of 1992/93 a pilot exposure assessment study was carried out to determine the distribution of environmental contaminants found in sport fish license holders, prior to the development of large-scale health effects studies. The study examined the relationship of fish and wildlife consumption with levels of persistent environmental contaminants and selected biomarkers found in blood, hair and urine samples.

A total of 232 people (fisheaters and controls) participated from two communities, Cornwall and Mississauga (out of 705 people contacted). Participation rates were lower than expected. Sport fish and wildlife consumption and potential confounder information was obtained using an interviewer administered questionnaire. Blood and hair samples were analysed for environmental contaminants including PCBs, organochlorines pesticides, mercury, lead, and cadmium. Pooled blood samples were analysed for dioxins, dibenzofurans and coplanar PCBs. Biomarkers including liver enzymes and thyroid hormones were measured in serum samples; cotinine, porphyrins and d-glucaric acid were measured in urine samples. All individual results were provided to each participant and their physician with individualized interpretation of results.

Cornwall participants primarily ate perch and walleye (pickerel) from the St. Lawrence River. Seventy six percent (76%) of Mississauga sportfish eaters ate fish from inland lakes and rivers; 40% ate fish from Lake Ontario. In Mississauga, salmon and rainbow trout were the most commonly eaten fish from the Great Lakes. Bass and walleye were the most commonly eaten fish from inland lakes and rivers. Fish eaters also ate more game and waterfowl than non-fish eaters in both communities.

The mean sport fish consumption rate (for eaters only) was 21.3 g/day (similar in both communities). The mean consumption rate for Great Lakes fish (for eaters only) was higher in Cornwall than Mississauga (19.9 vs 12.3 g/day). The consumption rates for all sport fish are similar to those of some other angler studies in the Great Lakes Basin but lower than the sport fish consumption levels seen in some immigrants, residents of the North Shore Gulf of St. Lawrence and the Arctic where local fish and game consumption is a more important part of the regular diet.

Blood levels of PCBs and organochlorine pesticides in the study population were also generally low compared with other studies of fish eaters and levels known to be associated with adverse health effects. PCB plasma levels (measured as Aroclor 1260) ranged from 0.74 - 23.0 μg/L (wet weight). The geometric mean level was 4.2 μg/L. PCB levels (on a

lipid basis) were significantly associated with waterfowl (duck/goose) consumption (yes/no) and Great Lakes fish consumption (number of kilograms of Great Lakes fish eaten in last 12 months and number of years eating sport fish), after adjustment for age and gender. Eight of the 232 participants had PCB levels in their blood plasma which were slightly over Health Canada guidance values. In some of these participants, Great Lakes fish consumption for many years may have resulted in these slightly elevated levels.

Levels of dioxins, dibenzofurans and coplanar PCBs in 14 pooled samples were also low, similar to other studies of people with low to moderate fish consumption. Levels appear to be most strongly associated with age. Dioxin and dibenzofuran levels were similar for Great Lakes fish eaters and non-eaters except in Mississauga among older participants. Coplanar PCB levels were generally higher in Great Lakes fish eaters than non-eaters.

Total mercury levels in whole blood were generally low, ranging from <2 to 17 ppb; these levels were also associated with sport fish consumption (yes/no), after adjustment for age and gender. Hair mercury (total) levels were generally very low, detectable in only 36% of samples (range: <1 to 8.4 ppm). All blood and hair levels (with the exception of the hair sample from one participant) were below Health Canada guidance values. Lead levels in whole blood were generally well below blood guidelines (except for one person); the range was <0.5 to 26.8 μg/dL. Lead was found to be significantly associated with sport fish consumption (yes/no and kilograms eaten in last 12 months) and smoking (yes/no) after adjustment for age, gender and community. The overwhelming determinant for cadmium levels (in whole blood) was smoking (number of cigarettes). Cadmium levels ranged from <0.2 to 0.9 μg/L for non-smokers and 1.0 to 14.6μg/L for smokers. Twenty five participants (all smokers) had cadmium levels above the U.S. Occupational Safety and Health Administration action level of 5 μg/L.

Urinary cotinine was confirmed to be an effective exposure biomarker for smoking. Levels of liver plasma serum enzymes, serum thyroid hormones, urinary porphyrins and urinary d-glucaric acid were not found to be associated with fish consumption. It is recommended that in future studies biomarkers only be considered when it is clear that the population is exposed and that the biomarker is specific to the exposure of interest.

In conclusion, levels of contaminants (with the exception of cadmium) were generally low compared with other studies of fish and wildlife eaters and below levels known to be associated with adverse health effects. Within these low ranges, PCBs, mercury and lead levels were found to be higher in fish eaters compared with non-eaters. However the magnitude of these differences and the number of people with elevated levels are not sufficient to conduct further health studies with the fish license holder population at this time.

2.0 ACKNOWLEDGEMENTS

We want to thank all of the people who have contributed so much to this project. Above all, we would like to thank the 232 participants from Cornwall and Mississauga who generously provided hair, urine and blood samples and gave of their time to answer a detailed questionnaire. In a study such as this, the personal gains to each participant are not large, except perhaps for the knowledge that they are contributing to the understanding of the link between environmental pollution and human health.

The project involved a number of stages and components. In order to be as complete as possible, we have attempted to acknowledge all of the people, organizations and companies involved in rough chronological order. We apologize for any omissions.

A number of people provided valuable input to the project planning, including Dr. Bourdeaux and other staff at the Eastern Ontario Public Health Unit, Paula D'allOsto and staff at the Peel Health Unit, Janette Anderson, Mike Eckersley and Murray German of the Cornwall Remedial Action Plan Team, Elaine Kennedy, John Milnes and Daniel Green of the Cornwall Public Advisory Committee, and Elliot Snitman, Phil Weir and Mark Tarnawczyk of the Credit River Anglers Association.

The mail-out Great Lakes Basin Anglers Study was carried out by SPR Ltd. (Toronto) for the Laboratory Centre for Disease Control and the Great Lakes Health Effects Division. The data were compiled for use in this study by Peter Walsh (Laboratory Centre for Disease Control). The Lancaster interviews were carried out under contract by Audrey Cumming (Lancaster), Johanna Feilotter (South Lancaster), and Monique Yates (Green Valley).

Valuable epidemiological and questionnaire advice was received from Dr. Tye Arbuckle. The dietary questionnaire and meal size methodology was developed under contract by Elizabeth Campbell-Assellbergs, St. Catharines. The fish sampling used for the length-weight regressions was carried out under contract by John Radford, Port Robinson. Sue Efler, a student in the Great Lakes Health Effects Division (Health Canada), produced the first draft of the protocol for collection, storage and shipping of samples. Dr. Rick Burnett and Dr. Cheryl Bartlett have provided statistical advice to the project. Mary Hegan, Betty-Ann Fry and Lynn Andrews (Great Lakes Health Effects Program, Health Canada) have provided advice regarding public consultation and communication activities.

Sue Cragg, Pam Baxter and Alex Turnbull of Goss, Gilroy & Assoc. (Ottawa) coordinated the field component of the study, including participant recruitment, public meetings, questionnaire focus groups, interviewer and nurse hiring and training, visit scheduling, questionnaire implementation and sample collection, sample processing and shipping and data keypunching. Many thanks to the nurses and interviewers in Cornwall (Shelley Kitts, Heather Lightfoot, Shelley Mallette, Karen Eitzen, Lori McDermid, Barbara Campbell) and Mississauga (Susan Winch, Nikki Baker, Cindy Stewart, Paramjit Rai, Sandi Martin).

A number of scientists have been involved in the analyses of the biological samples. Dr. Jean-Philippe Weber and Dr. Lilianne Ferron of the Centre de Toxicologie du Québec analyzed the organochlorine samples. The metals analyses were done by Medical Services Branch, Health Canada (Dr. Christine Gillette-Welling). The clinical chemistry and lipid analyses were performed by the Ottawa Civic Hospital (Dr. Sherry Perkins). Urinary porphyrin analyses were also done by Dr. Perkins for Mark Feeley and Dr. Don Grant (Foods Directorate, Health Canada). Dr. Raymond Poon (Environmental Health Directorate, Health Canada) carried out the urinary cotinine and d-glucaric acid analyses. Dr. Dave Blakey, Katherine C. Huang and Janet Bailey (Environmental Health Directorate, Health Canada) are doing the chromosomal abberation analyses. Dr. Jake Ryan (Foods Directorate, Health Canada) is doing the analyses of pooled plasma samples for dioxins, furans and coplanar PCBs.

The onerous tasks of data management and preliminary analyses were carried out by Robert Tkalec (Environmental Health Directorate, Health Canada).

Ongoing support for the project has been provided by Dr. Andy Gilman (Chief), and Dr. Dieter Riedel (Section Head), Great Lakes Health Effects Division, Health Canada.

Last, but not least, Francine Vinette has provided clerical and word processing support throughout the project.

3.0 INTRODUCTION

The most significant route of exposure to persistent environmental contaminants in the Great Lakes Basin is likely to be through the ingestion of sport fish caught from the Great Lakes/St. Lawrence River (Van Oostdam & Kagey 1991). Consequently, people consuming large amounts of contaminated fish are likely to have greater exposure to several persistent pollutants (Environment Canada et al 1991). Accurate assessment of human exposure is critical in the determination of the potential health effects of environmental contaminants found in the Great Lakes Basin. Various authors and organizations have recommended that exposure assessment must focus on assessing exposure of groups most at risk including sport fishermen and hunters (Steward & Olson 1991, Van Oostdam & Kagey 1991, Health & Welfare Canada 1991, Todd and Durkee 1989, International Joint Commission 1991).

The Great Lakes Health Effects Cohort Study was a multi-phased study planned to investigate the exposure and possible health effects due to increased exposure to environmental contaminants through consumption of contaminated fish and wildlife from the Great Lakes Basin. Under the Cohort Study, health effects research was planned using a cohort of 'high' fish consumers identified through the use of a "screening" mail-out questionnaire, mailed to a sample of Ontario sport fishing license holders. A planning workshop was held in March 1991 to begin to set priorities and the framework for the future health effect studies within the Cohort Study. It was concluded that before implementing the full Cohort Study, more information was needed 1) on the levels of exposure found in 'high' fish consumers and 2) on the proportion of the population that consume large numbers of contaminated fish (Health and Welfare Canada 1991).

The primary objective of this pilot exposure assessment study was to address the need to obtain further information on exposure, as measured by contaminant and biomarker levels in blood, hair and urine, in a group of "high" consumers in two communities, Mississauga and Cornwall and to refine the appropriate methodology. These two communities have active local fishing communities and have fish advisories that are in effect for some local species in each area.

4.0 BACKGROUND INFORMATION

4.1 Fishing activity in Ontario

Over 1 million fishing licenses are issued every year in Ontario to persons between the ages of 18-64. A 1985 mail-out survey of anglers by the Department of Fisheries and Oceans (DFO 1989) estimated resident fishing activity in the Great Lakes area at 12.8 million fishing days with an estimated 31 million fish caught and kept. For Lake Ontario there were an estimated 3.5 million fishing days in 1985 with an estimated 4 million fish caught and kept. For the St. Lawrence River there were an estimated 890,000 fishing days with an estimated 4 million fish caught and kept.

Data compiled from a number of different intensive boat surveys by the Ontario Ministry of Natural Resources estimated a total of 845,100 fishing days for L. Ontario in 1989 for a total of 612,800 fish (1,451,200 kilograms) harvested (Savoie & Bowlby 1990, Stewart et al. 1989). However the authors estimate that 53 percent of the Lake Ontario salmonid fishing effort has not been recently surveyed. Most of this recently unsurveyed activity is lakeshore and stream based (vs boat based).

A major activity in Lake Ontario until 1993 was the annual Toronto Star Great Salmon Hunt. The derby ran through July and August and attracted 15-20,000 entrants. A mail survey conducted in 1989 of 603 entrants indicated chinook salmon and rainbow trout were preferred species followed by coho salmon, brown trout and lake trout (Cox & Johnson 1990).

A 1982 survey of fishing activity in Lake St. Francis estimated 373,000 angler hours per year; in 1988 this estimate fell to 107,200 angler hours per year, perhaps partly due to weather (St. Lawrence RAP Team 1992). Harvest in the lake is predominantly perch (296,800 fish in 1988, 788,400 fish in 1982). No daily limit or season exists for perch from L. St. Francis and anglers are permitted to sell perch to local residents, stores and restaurants. This amounts to an estimated 25,000 kg of fillets or 400,000-500,000perch annually. Walleye, northern pike, smallmouth bass and panfish are also harvested from Lake St. Francis.

4.2 Fish consumption levels

Data on sport fish consumption in Ontario come from a variety of sources. Derby fish consumption estimates for Lake Ontario, Lake Huron and Georgian Bay salmonid anglers are available from a 1989 survey conducted by the Ministry of Environment (Cox & Johnson 1990). The (predominantly) mail-out survey was completed by participants of the Toronto Star Great Salmon Hunt (Lake Ontario), Chantry Chinook Classic (Lake Huron) and Owen Sound Salmon Spectacular (Georgian Bay) derbies. Overall, 21.2% of Lake Ontario derby anglers consumed no fish compared with 7.3% in the Lake Huron derby and 8.9% in the Georgian Bay derby. The most frequent consumption rate response for L. Ontario derby anglers was 1 meal per month. The mean number of fish meals/year was 21.1 for Lake Ontario derby anglers and the average meal size was 246 grams (14.2 g/day); 62.4% of respondents said they freeze salmon and trout for later consumption. The authors conclude that "based on consumption patterns, approximately one in every six Lake Ontario anglers would be considered to be at a higher risk because of increased contaminant intake". It may be argued that the derby participants are more avid anglers than non-derby participants and hence are likely to eat more fish. At the same time the derby participants may be more aware of the sportfish consumption guidelines and hence, may restrict their fish consumption more than non derby participants. Most respondents to this survey were male. The question still remains as to how many of the female spouses/partners and children are consuming fish brought home by the angler which exceed the consumption advisories for women of childbearing age and children.

Fish consumption estimates for all Ontario anglers are available from the 1992, 1989 and 1986 mail-back surveys included in the Guide to Eating Ontario Sport Fish (Cox et al 1993, 1990, 1987). Respondents include Great Lakes and inland anglers across Ontario. The response rates to these surveys were very low (eg. 494/10,000in 1992); the respondents may be more motivated and knowledgeable about fishing and contaminant issues than non-respondents. The mean meal size was 276 grams in 1992, 272g in 1989 and 295g in 1986. For 1992, the average sport fish consumer ate 30 meals/year which translates to 22.5 g/day. For 1989 the daily consumption estimate was 20.2 g/day; for 1986 it was 24.7 g/day.

The meal size questions in the derby and guidebook surveys both asked how much fish was eaten at a single meal, followed by a list of suggested weight responses (eg. 4oz, 8oz, 12 oz, 1 lb etc.). Some participants may have trouble knowing the weight of the serving. Dietary surveys, such as the Nutrition Canada survey, prefer to allow the respondent to choose between pictures or models of different meal sizes, and calculate the serving weight by multiplying by a density factor (P. Verdier, Foods Directorate, Health Canada, 1991 pers. commun.).

A recent study was carried out with 20 Vietnamese women aged 18-57 years in Hamilton, Ontario (Cavan & Cole, pers. commun.). An objective of the study was to develop a consumption questionnaire to determine seasonal consumption and nutritional benefits of locally caught freshwater fish. The mean yearly number of freshwater fish meals was higher when asked seasonally and then summed (47.7 (s.d. = 29.3)) than when asked yearly (38.6 (s.d. = 17.6)). The most popular meals were rainbow smelt, white bass, bullhead, drum and lake trout. The average meal size was 180 g (s.d. = 57.5); the average yearly mass of Great Lakes fish consumed was 8.25 kg/year (s.d. = 4.9) with a daily consumption rate of 22.6 g/day (s.d. = 13.4).

A "National seafood consumption study" has been conducted by Health and Welfare Canada (Market Facts of Canada 1991). The study used 3,815 monthly diaries completed by a Consumer Mail Panel, sampled from the general Canadian population. Overall 5% of 'seafood/fish' meals were 'self-caught'. The average fish meal size (fish of any source) was 148 grams (respondents were originally asked how many "cups" of fish they ate at each meal) and daily consumption for eaters was estimated at 22 g/day. The average meal size for 'self-caught' fish was not presented in the report. The study found that meal size increased with the weight of the consumer.

A recent survey of literature reporting fish consumption rates for Amerindian populations (Coad & Richardson 1995) estimated a mean rate of fish consumption (from 72 data sets including dietary and harvest surveys) of 91 g/day. This is significantly higher than the mean consumption rate (16.2 g/day) estimated for Ontario Amerindians, using mercury hair levels from 4327 adults and fish contaminant levels in the vicinity of 58 reserves in Ontario.

For risk assessment purposes, Health Canada's Food Directorate uses 40 g/day as an estimate of mean intake of fish and shellfish muscle tissues, for eaters only. The average daily intake for the Canadian general population (ie. eaters and non-eaters), for all saltwater and freshwater fish and shellfish was estimated at 10.43 g/day (Conacher et al 1989). The freshwater fish component was 1.16 g/day. A similar value of 1.26 g/day is used for a Canadian general population of freshwater fish consumption in the human health assessments under the Canadian Environmental Protection Act (Health Canada 1994).

4.3 Concentrations of contaminants in Great Lakes fish

Levels of many persistent toxic chemicals (including chlorinated organic compounds and heavy metals) in fish from the Great Lakes have decreased substantially since the 1970's (Environment Canada et al 1991). However fish consumption advisories are still in effect for many fish species throughout the Great Lakes (Ontario Ministry of the Environment 1991). These advisories apply mostly to long-lived top predators, such as lake trout, salmon and walleye, and to fish such as carp that inhabit contaminated nearshore environments (Environment Canada et al 1991). For example, lake trout consumption advisories were in effect at all sites tested in Lake Ontario in 1991 (Environment Canada et al 1991). Fish from Lakes Ontario and Michigan generally have higher contaminant levels than those from the other lakes (Environment Canada et al 1991). Lake trout from Lake Ontario have the highest levels of PCBs, DDT, mirex, dieldrin and dioxins in the Canadian Great Lakes (Environment Canada et al 1991). A recent publication describes contaminant concentration trends in Lake Ontario lake trout between 1977 and 1988 based on the data on whole fish collected by the Canadian Department of Fisheries and Oceans monitoring program (Borgmann and Whittle 1991). Mercury concentrations decreased steadily during the study period while DDE decreased rapidly from 1977 to 1980 but then remained relatively constant (1988 mean 0.12 μg/g). Mirex concentrations generally decreased but with much year-to-year variation (1988 mean 0.17 μg/g). Dieldrin concentrations decreased from 1978 to 1988 (1988 mean 0.09 μg/g) but chlordane concentrations remained relatively constant (1988 mean 0.09 μg/g). PCB concentrations gradually declined over the study period to present levels (1988 mean 2.54 μg/g). Generally levels of PCB, DDE and mirex in trout increased geographically in Lake Ontario from east to west.

In Lake St. Francis, part of the St. Lawrence River downstream from east of Cornwall, mercury and PCB levels in walleye have shown a steady decline since the 1970's (St-Lawrence Remedial Action Plan (RAP) Team 1990). Currently there are no consumption advisories for perch in L. St. Francis but there are some in effect for walleye and pike.

4.4 Effect of fish preparation and cooking methods on fish contaminant levels

Levels of *organochlorine* contaminants in fish can be affected by preparation and cooking methods that reduce fat levels (Armbruster et al 1987, 1988, 1989; Skea 1979; Stachiw et al 1988; Voiland et al 1991, Zabik et al 1979, 1982, 1993). For example, trimming of the fat along the dorsal section, lateral line and the belly of Lake Ontario brown trout reduced levels of fat by 61.8 percent, total PCBs by 45.6% and mirex by 44.2% (Voiland 1991). Cooking methods that let the fat drip off the meat also reduce organochlorines (Armbruster et al 1989, Zabik et al 1979). Cooking methods do not affect mercury levels (Armbruster et al 1988).

4.5 Relationship between fish consumption and human blood contaminant levels

Previous studies have looked at the relationship between fish consumption and environmental contaminant levels in the body. A pilot study initiated in 1973 examined fish consumption and levels of PCBs in blood serum of 158 persons from 10 Lake Michigan shoreline communities (Humphrey 1983, 1988). The group was comprised of 90 people who consumed between 24 and 270 pounds of Great Lakes salmon and trout per year ('exposed') and 68 control participants who consumed less than 6 pounds/year. The 'exposed' group had serum PCB concentrations significantly higher (25-366 ppb[1]) than the 'control' group (<5-41 ppb). In 1979-1982 the earlier study group was expanded to 991 participants, 572 persons who consumed more than 26 pounds of sport-caught fish per year, and a matched comparison group of 419 persons who ate less than 6 pounds per year (Humphrey 1983, 1988). Annual fish consumption rates were measured over two seasons by field visits, interviews and dietary logs. PCBs, DDT, and DDE were detectable in nearly every fish eater. The ranges of concentrations were lower and median concentrations of each contaminant were significantly lower in the control group than the fish eater group (6.4 vs 19.6 ppb for total PCBs (as Ar1260); 10.5 vs 26.4 ppb for DDT, 9 vs 21.7 ppb for DDE). Other contaminants (HCB, β-BHC, oxychlordane, heptachlor epoxide, trans-nonachlor, dieldrin and mirex) were detectable in less than 50% of fish eaters and seldom in the control group. Improvements in the analytical quantitation of PCBs account for the apparent difference between median serums levels for participants in the pilot study in 1973 and later cohort study in 1979-1982 (Humphrey 1988).

In 1989 the Michigan cohort was reexamined (Hovinga et al, 1992, 1993). Levels of blood lead and cadmium and serum PCB and DDT were measured in samples from 115 Great Lakes fish eaters and 95 non-fisheating controls who had participated in the earlier study in 1979-82. Total serum DDT represented the sum of serum p,p'-DDT and 1.114 times the serum content of p,p'-DDE. The detection limit for PCB was 3 ppb; for DDT 1 ppb. Again mean PCB levels and DDT levels were significantly higher in fisheaters than controls (PCBs-19.0 vs 6.8μg/L;DDT-15.6 vs 6.8μg/L). Historic fish consumption was the most important predictor of serum PCB and DDT levels. The primary predictors for lead and cadmium were smoking and self-reported occupational and recreational exposure; the number of current annual fish meals was a significant but less important predictor for both lead and cadmium.

In 1979 a study was conducted in Triana, Alabama of 499 rural black residents who were exposed to DDT via consumption of contaminated fish taken downstream from a defunct

---

[1] For comparison purposes, 1 ppb = 1 μg/L = 1 ng/mL

DDT manufacturing facility (Kreiss 1985). Mean fish consumption prior to extensive publicity about the study was 4.3 fish meals per month. Total DDT levels in serum ranged from < 1 to 2820 ng/mL (ppb) (geometric mean of 75 ng/mL) compared with a general population geometric mean of 22 ng/mL for the general US population. PCB levels were analyzed in 458 serum samples; levels ranged from 3.2 to 158 ng/mL (geometric mean was 17.2 ng/mL). Thirty-six percent of the study population had serum PCB levels higher than 20 ng/mL.

A study looking at offspring of women who consumed Lake Michigan fish found that three measures of fish consumption were correlated with PCB levels in maternal serum samples (taken after delivery) and breast milk samples (Schwartz et al 1983, Jacobson et al, 1983). The study included 242 women each of whom had eaten at least 26 pounds (11.8kg) of Lake Michigan contaminated fish within the previous 6 year period. The control group consisted of 71 women who reported consuming no Lake Michigan fish in the past or during pregnancy (for a total of 313 participants).

Fish consumption was measured in the study in three ways 1) highest annual rate of Lake Michigan fish consumption; 2) rate of consumption during pregnancy; and 3) cumulative lifetime consumption. Each fish meal was assumed to consist of 230 g of fish. Fish consumption was converted to a PCB index for each species as follows:

PCB-kg per year/species = (number of fish meals/year) x (.23 kg fish/meal)
x (weighting factor for level of contamination for each species)

The weighting factors ranged from 1.0 for chinook salmon, coho salmon, lake trout, carp, catfish and bullhead; 0.6 for pike and pickerel down to 0.1 for smelts, whitefish and chubbs. This PCB-kg/year index was summed across species for each participant to calculate each woman's annual 'contaminant-weighted' fish consumption rate. The mean annual rate of PCB-fish consumption at the highest levels reported by the exposed mothers was between 1.2 to 41.7 PCB-kg/yr, with a mean rate of 6.7 PCB-kg per year. The women reported eating Lake Michigan fish for an average of 16.1 years (range = 1-40 years) and in the year preceding the infant's birth, mean level of fish consumption was 4.4 PCB-kg (range = 0-26.5kg).

PCB levels were measured (as Aroclor 1260 standards) in serum samples from 196 of the 313 participants. It is not clear how many of the 196 were fisheaters and controls. Of the 196 samples, 44 (22%) were 'detectable but not quantifiable'. No details are given as to which participants have missing samples or how the non-quantifiable samples were handled in the subsequent analysis. Serum PCB levels, quantified with the Aroclor 1260 standard, averaged 5.5 ng/mL for the whole group (6.1 (s.d. = 3.7) for fisheaters; 4.1 (s.d. = 2.7) for controls). The PCB serum levels increased at each additional level of contaminated fish consumption.

In 1985 a questionnaire survey was sent to 1600 Wisconsin anglers living in 10 counties (Fiore et al 1989). The mean annual number of sport-caught fish meals was 18 meals (including anglers with zero consumption). The mean number of non sport-caught fish meals was 24 per year. Assuming an average meal size of 227 g (8 oz) the mean daily sport-caught fish intake was 12.3g for fish consumers; the mean daily intake from all fish sources was 26.1 g. Seventy-two percent of respondents were familiar with the Wisconsin fish consumption advisory. PCB levels were determined in 192 blood samples by summing quantified values for 13 congeners (treating nondetectable values as zero). Of the 192 study subjects, 31% had no detectable levels of PCBs; the mean PCB congener sum was 2.2 μg/L and the median value was 1.3 μg/L (Fiore et al 1989). The congeners most frequently identified at quantitative levels were (in order from least to most frequent): 118 (2,4,5-3',4') (34% of samples), 180 (2,3,4,5-2',4',5') (42% of samples), 138 (2,3,4-2',4',5') (56% of samples), and 153 (2,4,5-2',4',5') (78% of samples) (Sonzogni et al, 1991). Analyses conducted using Aroclor standards consistently resulted in higher total serum PCB values. The mean PCB serum levels increased with increasing number of fish meals. The total number of sport-caught fish meals and kilograms of fish caught and kept for eating were both positively correlated with the PCB congener sum ($p = 0.003$ and $p = 0.0007$ respectively) (Fiore et al 1989). Eighty nine (46%) of the 192 samples had nondetectable DDE levels. The mean DDE level was 6.3 μg/L; the median was 5.4 μg/L and the range was nondetectable to 40.0 μg/L. There was also a statistically significant positive correlation between DDE serum levels and the total number of sport-caught fish meals ($p = .04$) but there was no significant correlation between DDE serum levels and the total kilograms caught and kept for eating.

Another Wisconsin study recruited pregnant women during their first prenatal visit (Dar et al 1992). Participants completed a self-administered questionnaire including questions on fish consumption. Based on the amount and species of fish reported and PCB levels from Department of Natural Resource surveys, a PCB exposure score was estimated. These intake scores were then used to stratify the population into three strata: low, consuming no locally caught fish ($n = 522$); medium, with PCB intake scores from 0 to the 90th percentile ($n = 401$); and high, for scores above the 90th percentile ($n = 104$).

Subsequently, 106 serum were randomly selected across exposure strata and analysed for DDE and 13 PCB congeners. Detectable PCB congeners (detection limit = 0.6 μg/L) were found in 24 samples and DDE in 10 (9/10 sera also had PCB congeners). Of the 13 analysed, five congeners were found: 118 (2/24), 138 (18/24), 153 (24/24), 180 (7/24) & 187 (1/24). The quantities ranged from the detection limit to 5.0 ppb. Correlation between the total PCB level in serum (wet weight sum of congeners) and "variables of local fish consumption" were reported to yield a Pearson correlation of 0.666. However, it is unclear which population of mothers was included in this analysis, whether the fish consumption score or the derived PCB index was used and what was done with non-detectable values. Judging from the calculation of mean congener levels, based only on the detectable values, it might be concluded that this correlation analysis was based on the 24 detectable levels which would severely limit the applicability of the analysis result to the entire prenatal population.

Almost 200 members of Gulf of communities along the St. Lawrence Lower North Shore took part in a study of the risks and benefits of fish, seafood, wildlife and sea bird egg consumption (Dewailly et al 1991). Questions were asked on demographics, consumption patterns and medical history. The mean fish consumption estimate (including marine mammals) was 524.8 g/week (ie. 75 g/day); the mean seabird consumption was 188.2 g/week (ie. 26.9 g/day). Contaminants measured in blood included metals (lead, cadmium and mercury), PCB's, as Aroclor 1260 and congeners, and other organochlorines. Total mercury levels in whole blood ranged from 4-106 nmol/L (geometric mean of 27), with only 3.5% non-detectable (detection limit = 10 nmol/L). Mercury levels increased with increasing consumption of fish, sea birds and bird eggs. PCBs, measured as Aroclor 1260, serum levels ranged from 1.4-160 μg/L with an arithmetic mean of 35.2. Seven congeners were detected in the majority of samples (detection limit = 0.2ug/L): 118 (89%), 138 (100%), 153 (100%), 170 (88%), 180 (99.5%), 183 (77%) & 187 (93%). Arithmetic means of plasma levels of these congener levels ranged from 0.36-3.86 μg/L. Addition of the congeners resulted in estimates of totals about 35% of Aroclor 1260 levels. Other organochlorines detected (detection limit = 0.2 μg/L) included: DDE & HCB (100%), mirex (96%), dieldrin (60%) and γ-chlordane (1%). No heptachlor was detected. Arithmetic mean levels calculated on all values, with substitution of 1/2 detection limit (i.e. 1ug/L) for non-detectables, ranged from 0.23 μg/L for dieldrin to 16.4 μg/L for DDE. Higher Aroclor 1260 levels were associated with annual seabird egg consumption and weekly fish consumption.

In summary, a number of studies have found relationships between consumption of fish and wildlife (measured in a variety of ways such as kg sportfish/year, number of sportfish meals, PCB exposure indices, cumulative lifetime consumption) and levels of environmental contaminants in humans (including PCBs, DDE, other OCs, mercury, cadmium and lead).

5.0 STUDY OBJECTIVES AND PRIMARY HYPOTHESIS

The *primary objective* of this study was *to investigate the distribution of environmental contaminant levels found in blood and hair of people consuming fish and wildlife from presumably high consumption areas of the Great Lakes/St. Lawrence River in Ontario* in order to:

- compare levels of environmental contaminants found in the participants of this study with those found in other selected populations
- compare levels of environmental contaminants found in the participants of this study with levels of toxicological concern
- estimate the difference in levels of selected contaminants in blood and hair between Great Lakes fish consumers and non-consumers
- assess whether the magnitude and difference of these levels warrants health effects studies
- decide whether the population of fish licence holders with elevated levels of persistent environmental contaminants is sufficiently large to conduct health effects studies.

Secondary objectives of the study were to:

- assess the feasibility of using a cohort of fish licence holders to recruit high fish consumers for exposure and health effect studies.
- to determine the relationship between reported fish and wildlife consumption, as measured by interview-administered questionnaires, and contaminant levels measured in blood and hair.
- to examine the distribution of selected biomarkers in fish consumers and non-consumers and to assess the contribution that selected biomarkers could make as measures of pre-clinical health effects in future studies of populations exposed to environmental contaminants

The *primary null hypothesis* of the study was:
There is no difference in contaminant body burdens as measured by blood and hair levels in people consuming fish from the Great Lakes basin as compared to non-consumers.

6.0 METHODS

6.1 Study Sites

The study was conducted in the Cornwall area and city of Mississauga in Southern Ontario (Figure 6-1). These sites were chosen because of their active fishing communities and their proximity to fishing areas where consumption guidelines are in effect for some fish species. As well, the Cornwall Remedial Action Plan team was interested in obtaining more information about local fish and waterfowl consumption. The two communities are defined below:

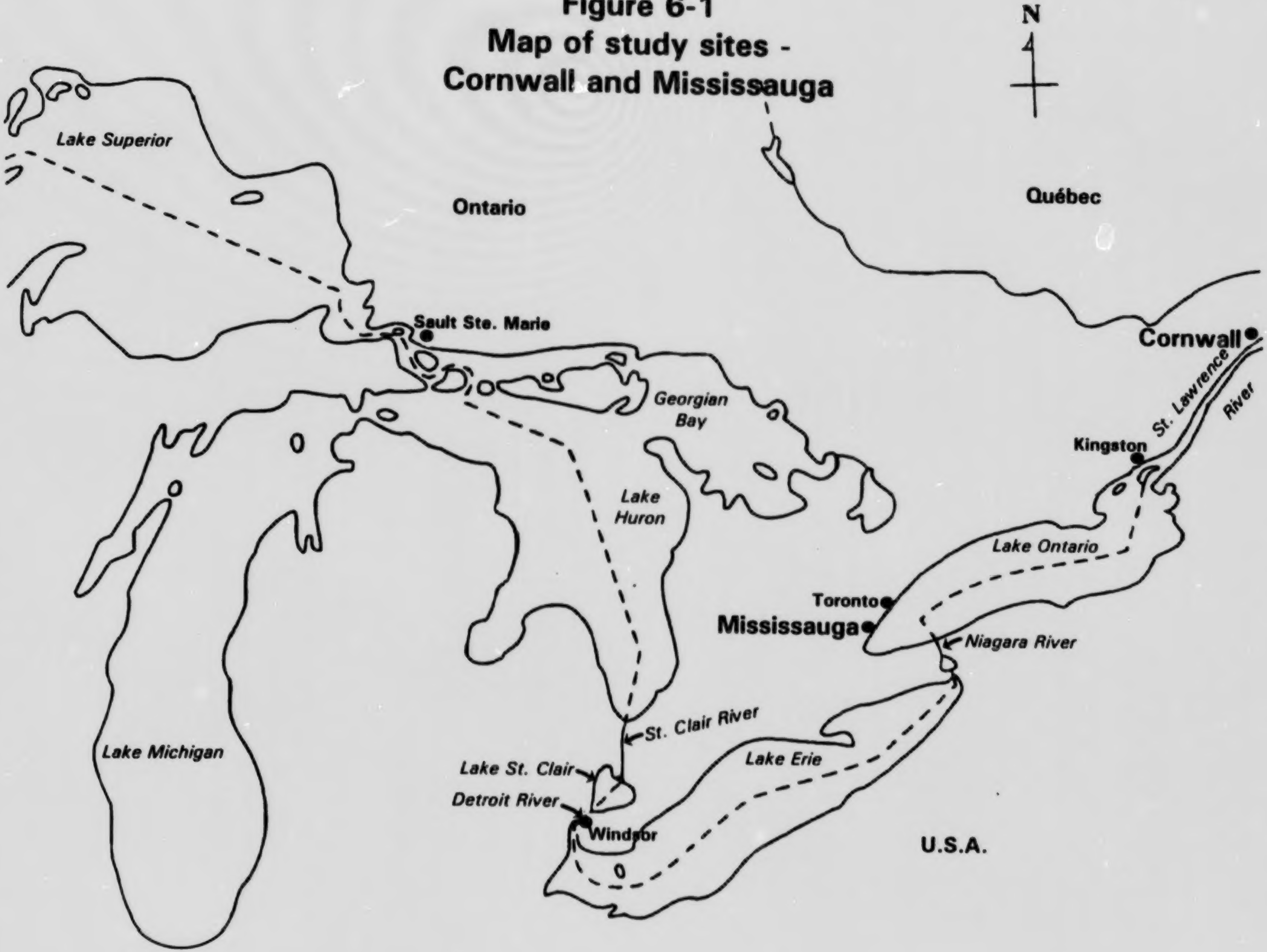
A. Cornwall area: Stormont, Dundas and Glengarry United Counties (Census Division 01), **excluding** Lancaster Township (Geocode 01 001), Lancaster Village (Geocode 01 003), Charlottenburgh Township (Geocode 01 006), and Akwesasne 59 Reserve (Geocode 01 007).

B. Mississauga: The city of Mississauga (Geocode 21 005) in Peel Regional Municipality.

The Cornwall area covering Stormont, Dundas and Glengarry counties had a population of 102,000 (1986 census). The St. Lawrence River is a very popular fishing area both in Lake St. Lawrence (upstream of the Moses-Saunders Dam) and Lake St. Francis (downstream of the dam), particularly near the community of Lancaster. Between 300,000 and 750,000 yellow perch are taken from the Ontario side of the lake every year (Ontario Dept. of Environment 1991). There are 3 commercial fishermen operating in Lake St. Francis. There are no consumption advisories for perch from Lake St. Francis and there are no catch limits. Perch are sold throughout the community by local anglers, small stores and restaurants. Mercury and PCB levels have resulted in consumption guidelines for Lake St. Francis for walleye (>45 cm), northern pike (>45 cm), sturgeon, channel catfish, sucker (>45 cm) and smallmouth bass (>35cm). The Mohawks of Akwesasne have advised their community to eat no fish from the St. Lawrence River. There is a small amount of data on concentrations of contaminants in waterfowl. Mallards collected from Lake St. Francis in 1988 and 1989 had the highest PCB levels in the Great Lakes Basin (mean 48.04 ng/g wet weight (St Lawrence Remedial Action Plan Team, 1990)).

Mississauga, a city within Peel Region, has a population of 446,000. It is located on the shore of Lake Ontario and has been called the "Salmon fishing capital of Canada". It is home to the largest sport fishing boat fleet on the lake. The Credit River, located in the middle of Mississauga, attracts thousands of anglers during the salmon run. Mirex and PCB levels have resulted in consumption advisories in some locations in Lake Ontario and the

Figure 6-1
Map of study sites -
Cornwall and Mississauga


N
Lake Superior
Ontario
Québec
Sault Ste. Marie
Cornwall
St. Lawrence
River
Georgian
Bay
Kingston
Lake
Huron
Lake Ontario
Toronto
Mississauga
Niagara River
St. Clair River
Lake Michigan
Lake Erie
Lake St. Clair
Detroit River
Windsor
U.S.A.

Credit River, particularly for larger lake trout, brown trout, chinook salmon, catfish, walleye, and eel (MOE 1991).

6.2 Public consultation and information

The study objectives and implementation methods were discussed with various agencies and groups. In Cornwall, the study team collaborated with the Remedial Action Plan (RAP) team, the Public Advisory Committee and the Eastern Ontario Public Health Unit. The area surrounding Cornwall from the Moses Saunders power dam to the eastern end of Lake St. Francis is one of 43 "Areas of Concern" in the Great Lakes-St.Lawrence ecosystem. A Remedial Action Plan (RAP) is being developed to restore and protect water quality in this stretch of the St. Lawrence River. The RAP team, with representatives from Environment Canada, the Ontario Ministry of Environment and Energy, the Ontario Ministry of Natural Resources, the Mohawks of Akwesasne and a Public Advisory Committee are working with other federal, state and provincial agencies to co-ordinate the RAP development and implementation. One of the interests of the RAP team has been the collection of local fish and waterfowl consumption data.

In Mississauga discussions about the study were initiated with the Peel Regional Health Unit and the Credit River Anglers Association.

Further discussions occurred with the Advisory Committee to the Great Lakes Health Effects Program, and with participants of a Great Lakes Health Effects Program public consultation workshop, held at Jackson Point in collaboration with Great Lakes United in July, 1991.

Information about the initial mail-out survey was prepared and distributed in a number of ways. Fact sheets were prepared for Cornwall and Mississauga. They were distributed in Cornwall by the Environmental Resources Center, at a shopping mall display in November, 1991 and by the Public Advisory Committee at the Cornwall Sportsmen's show in March, 1992. They were also sent out to all people on a mailing list for the St. Lawrence Remedial Action Plan team.

A notice about the study was included in the November issue of "Call of the Loon", the monthly newsletter of the Federation of Ontario Anglers and Hunters (FOAH). The same notice was included in the winter issue of "Angler and Hunter", the magazine of the FOAH.

A handout on environmental contaminants in fish, "Having your catch and eating it too", was prepared and distributed for background information.

To deal with local physician concerns and to enlist their support, the study's medical advisor, in collaboration with the local public health units, did presentations on environmental health, fish consumption and the purpose of the study at local hospitals in Cornwall (1) and Mississauga (2).

Information provided to the individual participants is described in Secton 6.4, including an information meeting held in each community for potential participants. The study was committed to providing a summary of individual analytical results to each participant and their physician (if desired). The study protocol was approved by the Human Ethics Committee, Health Protection Branch (Health Canada) in June 1992.

A handout describing the study results is being prepared and will be distributed to the participants and other community members.

6.3 Population/sampling strategy

The sampling frame for biological sampling (Stage 2) was constructed from the group of people who responded to a mail-out questionnaire "The Great Lakes Basin Anglers Survey" (Stage 1, see Table 6-1).

Table 6-1
Framework for study implementation

Stage	Purpose	Implementation	Output	Time Frame
1	Create sampling frame for stage 2	• Great Lakes Basin Anglers Survey questionnaire mailed to 2000 fish license holders in each community • 94 personal interviews in Lancaster and 350 mail out surveys	• list of anglers with basic information on fish consumption	Nov'91 - Mar '92
2	Biological sampling and dietary questionnaire	• identified fish consumers and non-consumers • solicited 232 participants • conducted biological sampling and interviews • conducted laboratory analyses • disseminated individual results	• information on (1) levels of environmental contaminants and biomarkers in participants and (2) fish consumption patterns	Nov '92 - July '93

This questionnaire was mailed out in winter 1991/92 to a random sample of fish license holders in both communities (with oversampling for women). A small number of people in the Lancaster area (near Cornwall) completed the questionnaire by face-to-face interview. A flow chart depicting the sampling process is given in Figure 6-2. The next section (6.3.1) provides a brief summary of the implementation of this questionnaire (mail-out and Lancaster interviews). Section 6.3.2 describes how the sample frame for the biological sampling was subsequently created.

6.3.1 Stage 1 - Methods used in mail-out questionnaire and Lancaster interviews

Mail-out questionnaire

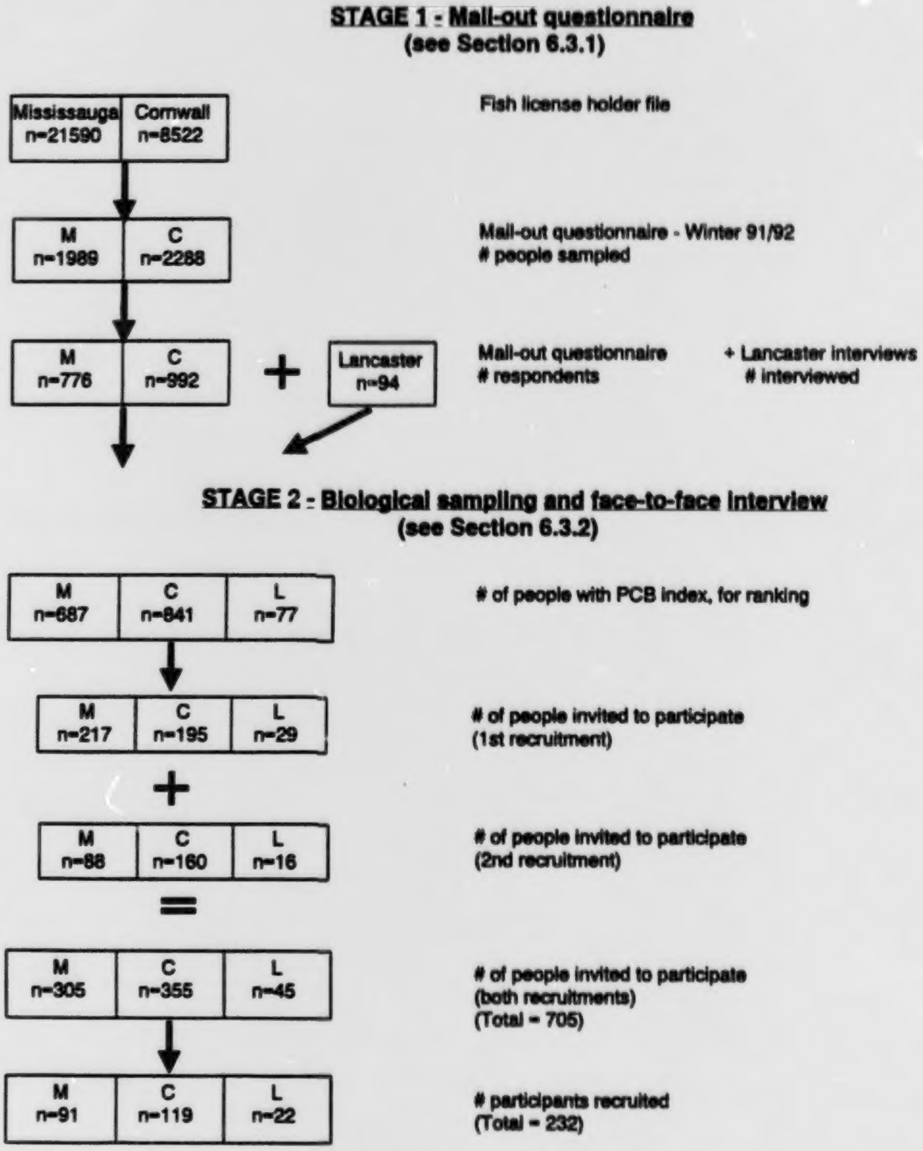
The mail out questionnaire was implemented by the Laboratory Center for Disease Control, Health and Welfare Canada, under contract to SPR Ltd. Toronto (GLHEP Project #10). The "Great Lakes Basin Anglers Survey" was mailed to approximately 4000 sport fish license holders in Cornwall and Mississauga during winter 91/92. The questionnaire contained basic questions on fish consumption, lifestyle factors and general health (see Appendix 1).

The sampling frame for this mail-out consisted of all people living in the study areas who were in a file of 1988 sport fishing license holders. This file was created by the Laboratory Center for Disease Control of Health Canada, in co-operation with the Ontario Ministry of Natural Resources. License information included name and address of license holder, location of purchase of license and birth date. Gender was imputed using the first name in 91.3% of the original records. Addresses in this file were updated by linkage to the Ontario Ministry of Transportation 1991 Drivers License file.

A breakdown of the number of people in the license file by gender and age category for each community is given in Table 6-2. A stratified random sample of approximately 2000 licence holders was selected for each community based on age and gender, with over sampling for women, as given in Table 6-2. Distribution and collection of the questionnaires and data entry was carried out by SPR Ltd Toronto under contract to the Laboratory Center for Disease Control, Health Canada (SPR, 1991b).

The questionnaire package included a covering letter, return envelope and questionnaire. It was followed a week later by a thank you/reminder postcard mailed to all individuals in the survey. Approximately 3 weeks after the first mailing, a second questionnaire package was sent out which included a letter reminder, return envelopes and the survey. This package was sent to all individuals who had not returned their Great Lakes Basin Anglers Survey. English and French "collect call" telephone lines were maintained by the contractor to answer questions and concerns. A "hot-line" manual was prepared by the contractor which outlined how enquiries were to be answered. A log of questions and answers was kept.

Figure 6-2
Sampling Process


STAGE 1 - Mail-out questionnaire
(see Section 6.3.1)
Mississauga n=21590
Cornwall n=8522
Fish license holder file
M n=1989
C n=2288
Mail-out questionnaire - Winter 91/92
# people sampled
M n=776
C n=992
+
Lancaster n=94
Mail-out questionnaire
# respondents
+ Lancaster interviews
# interviewed
STAGE 2 - Biological sampling and face-to-face interview
(see Section 6.3.2)
M n=687
C n=841
L n=77
# of people with PCB index, for ranking
M n=217
C n=195
L n=29
# of people invited to participate
(1st recruitment)
+
M n=88
C n=160
L n=16
# of people invited to participate
(2nd recruitment)
=
M n=305
C n=355
L n=45
# of people invited to participate
(both recruitments)
(Total = 705)
M n=91
C n=119
L n=22
# participants recruited
(Total = 232)

The data were coded and entered with 100% verification. Range and logic tests were conducted during data capture, and inconsistencies in responses were flagged. A data processing strategy and edit-code protocol was prepared by the contractor.

Table 6-2
Sampling frame and response rates for mail-out questionnaire

Community	Gender	Age[1] Category	# of license holders	# of license holders sampled	# of respondents (and percentages) to mail-out questionnaire
Cornwall	Female	18-39 yrs	1051	578	193 (33%)
		40-64 yrs	918	577	271 (47%)
	Male	18-39 yrs	3510	579	239 (41%)
		40-64 yrs	3043	565	289 (51%)
	TOTAL		8522	2288	992 (43%)
Mississauga	Female	18-39 yrs	2097	497	160 (32%)
		40-64 yrs	1194	496	214 (43%)
	Male	18-39 yrs	10497	499	164 (33%)
		40-64 yrs	7802	497	238 (48%)
	TOTAL		21590	1989	776 (39%)
OVERALL TOTALS				4277	1767 (41%)

[1] Age calculated as of Jan1/91.

Lancaster interviews

On the advice of the Eastern Ontario Public Health Unit, in the Lancaster area (Lancaster Township (Geocode 01 001), Lancaster Village (Geocode 01 003) and Charlottenburgh Township (Geocode 01 006)), three local health professionals conducted personal interviews with community residents. The Health Unit staff suggested that this might be a more effective way to reach high fish consumers in the area. The interview team worked in the community to identify people based on their fish consumption habits, not their fishing habits. This included people who may or may not have had a fishing licence in 1988, i.e. they may or may not have been in the sampling frame for the mail-out survey. A mail-out survey of 358 license holders also occurred in the Lancaster area (Geocodes 01 001, 01 003 and 01 006).

The interviewers were originally asked to seek out and interview non-native individuals who have eaten 26 or more meals of fresh-water fish in the past 12 months ('high' consumption group). The interviewers had difficulty in finding people consuming fish this frequently, so this criterion was dropped to 12 or more meals of fresh-water fish in the past 12 months. The freshwater fish could come from any source including stores and restaurants, or could be caught by the individual or other people. The fresh-water fish could have been previously frozen or otherwise preserved, or obtained fresh. The interviewers attempted to match a non-fish consumer with each "high" consumer. A non-consumer was considered to be someone who did not eat any kind of fresh-water fish from any source. A non-consumer was matched to a "high" consumer on gender, smoking status and age (within 5 years). Strata were defined by combinations of 3 age categories, 2 smoking categories and 2 genders. The age categories were 18-39 years, 40-59 years and over 60 years. Smokers were defined as people currently smoking 1 or more cigarettes, cigars, or pipe-fulls per day. Non-smokers were people who had not smoked a pipe, any cigarettes or cigars during the past 5 years. The interviewers attempted to identify 6 "high consumers" in each of 12 strata.

Interviewer notes describing the sampling strategy were prepared for the interviewers who were nurses living in the Lancaster area community. They attended an interviewing training session conducted by Opinion Search Ltd. which covered basic interview techniques. A "first contact questionnaire" was used to establish whether a person met the criteria to participate and whether they were willing to be interviewed. Using this information, the interviewer determined the strata that the individual belonged to and, if appropriate, established a convenient time and date to interview the individual. A total of 94 interviews were conducted. Table 6-3 gives the number of people interviewed in each stratum. All questionnaires were sent to Health Canada and the data were entered by the contractor handling the mail-out phase (SPR Ltd.).

Table 6-3
Number of Lancaster interviews

Gender	Age[1] category	Fish consumers		Non-fish consumers		Total
		Smokers	Non-smokers	Smokers	Non-smokers	
Female	18-39	3	4	3	6	16
	40-59	6	6	1	4	17
	>60	2	6	1	8	17
Male	18-39	5	3	2	3	13
	40-59	7	8	1	3	19
	>60	3	6	0	3	12
TOTALS		26	33	8	27	94

[1] Age as of Jan 1/91.

6.3.2 Stage 1 - Creation of sampling frame for biological sampling

The sampling frame for the biological sampling was based on the 1767 respondents to the mail-out questionnaire (776 in Mississauga, 991 in Cornwall) and the additional 94 interviews which were carried out in Lancaster. The 94 respondents to the Lancaster interviews were included with the Cornwall respondents to form the Cornwall sampling frame. The plan was to sample 224 participants in total (112 participants in each community - 56 controls, 56 exposed). This number of participants was chosen to provide reasonable power to detect differences in contaminant levels between the "exposed" and "control" groups. Initially a 50% response rate was assumed. Therefore an initial identification of 448 potential participants (112 exposed + 112 controls in each community) was required.

For each member of the sampling frame (n = 1767) a total PCB intake from sport fish in the last 12 months was estimated. This measure, called a "PCB index" was used as a relative measure to rank individuals in order to define the "exposed" and "control" groups. Total PCBs was chosen as an indicator chemical for exposure to persistent toxic chemicals from sport fish in the Great Lakes. The PCB "index" was calculated for each individual using the reported number of sport-caught fish meals consumed in the last 12 months (question 6) and the species-location information (question 2) from the Great Lakes Basin Anglers Survey, combined with average PCB residue levels in fish from that location provided by the Ministry of the Environment (see Figure 6-3). Information on the distribution of the 'PCB index' is provided in Table 6-4. It is interesting to note that the mean PCB index (of the non-zero values) is similar in Cornwall and Mississauga, indicating that the distributions of estimated PCB intakes from fish were similar in the two communities. Examination of the results of the mail-out indicated that Cornwall fisheaters ate more fish but less contaminated species than Mississauga fisheaters, resulting in similar PCB index distributions.

Within each community all respondents for whom a PCB index could be calculated, were ranked according to the PCB index. The 112 respondents with the highest PCB index in each community were selected as the "exposed" group. The control group was defined as those people who had a PCB index of 0 and met each of the following 3 criteria:

- ate no sport-caught fish or wildfowl or turtles in the last 12 months.
- ate no more than 3 freshwater fish meals in a restaurant, from a store or purchased from an individual in the last 12 months (question 7).
- never ate sport-caught fish or have not eaten it since 1985 (question 4).

In Mississauga this selection process resulted in a potential control group of 105 respondents. The Cornwall/Lancaster potential control group consisted of 200 individuals, out of which 112 respondents were randomly selected.

Figure 6-3
Calculation of "PCB index"

- The total number of sport-caught fish meals in the last 12 months was calculated using Question 6.
- Question 2 provided information on numbers, species and locations of fish caught and kept for eating by the household. Using the average weight of each species provided by the Ministry of Environment, (based on records of fish caught in the area) the total weight of the catch was calculated. A "percentage of the total catch" was calculated for each species/location combination.
- These percentages were applied to the total number of meals to estimate the total number of meals from each species/location.
- Using an estimated 227 g/meal (from Humphrey's studies) the PCB intake was estimated for the number of meals from each species/location. The 227g/meal estimate was considered to be in the mid range of values reported in the literature.
- The estimated PCB intake from each species/location was summed over all meals to provide an estimated total PCB intake from sport-caught fish in the last 12 months.

For example, if a person responded to question 6 that they ate 12 meals in the last year and responded that the household caught and kept for eating 5 walleye from the St. Lawrence River and 40 perch from the St. Lawrence River:

5 walleye @ 1700 g =	8500 g
40 perch @ 240 g =	9600 g
	18100 g

Walleye comprises 8500g/18100g = 47% of the catch by weight, and perch comprises 53 % of the catch. It was assumed then that of the 12 meals, 5.64 meals were walleye and 6.36 meals were perch from the St. Lawrence River.

The average PCB contaminant levels (provided by Ministry of Environment) for these species from the St-Lawrence River near Cornwall are 172 ppb (walleye) and 10 ppb (perch). Therefore,

for walleye: 5.64 meals x .227 kg/meals x 172 μg/kg = 220.21 μg
for perch: 6.36 meals x .227 kg/meals x 10 μg/kg = 14.43 μg
234.64 μg PCB's.

The PCB index is therefore 234.64 μg/yr.

Table 6-4
'PCB index' distribution

	Cornwall	Lancaster interviews	Mississauga
Number of zero values	384 (39%)	34 (36%)	314 (40%)
Number of non-zero values	457 (46%)	43 (46%)	373 (48%)
Arithmetic mean (median) (μg/yr)	476 (147)	890 (293)	474 (98)
Maximum (μg/yr)	10470	5039	8277
Number of missing values[1]	151 (15%)	17 (18%)	89 (12%)
Total number of respondents	992 (100%)	94 (100%)	776 (100%)

[1] unable to calculate PCB index due to missing information

Because the participation rate in the initial recruitment was lower than had been predicted (30-40% range), it was decided to enlarge the groups by including the next 88 "exposed" and 88 "control" people in each area, the numbers estimated to obtain the required numbers of participants. This second recruitment was not possible for Mississauga controls as all of the 105 respondents that met the 'control' criteria were sampled in the first recruitment.

6.4 Field methods

Field work including recruitment of participants, biological sampling and questionnaire interviews, processing and shipping of samples was carried out by a consulting firm, Goss Gilroy Inc. In each community a study coordinator and field teams operated from a study office which had phone, fax, and refrigerator facilities. Three field teams, consisting of one nurse and one interviewer, were hired in each community. A report on all field methods (including training activities) was prepared by Goss Gilroy Inc. (1993).

6.4.1 Recruitment of participants including informed consent

All potential participants for the biological sampling stage of the study were contacted in writing. A follow-up phone call was made a week later to

- confirm that they had sufficient ability to understand English or French
- confirm that they had no medical conditions that precluded blood sampling
- answer any questions from the potential participant
- remind them of the information meeting

If an individual was interested in participating at that time, more information about the study was sent to them, including a consent form (see Appendix 2). The participants were asked on the consent form if they would like their individual results sent to their physician. This was encouraged to improve the interpretation of the analytical results for the participants.

An information meeting for potential participants was held in each community to describe the purpose and methods of the study, and to answer any questions. A second phone-call was then made to all individuals who had neither agreed to nor refused to participate. Upon receiving the completed consent forms, the participants were contacted again to schedule a meeting in their home or another convenient location. Information on fasting requirements and urine sample containers were provided before the appointment.

After the interview participants were given a copy of the Guide to Eating Ontario Sport Fish and a poster of 'Fish of Ontario' produced by the Ministry of Natural Resources.

6.4.2 Questionnaire instrument and implementation

An interviewer-administered questionnaire (Appendix 3) was used to obtain more detailed information on the consumption of contaminated fish and wildlife, in order to predict exposure to contaminants through consumption of sport-caught fish and wildlife. The questionnaire was administered face-to-face by a trained interviewer after the blood sample had been taken. It contained questions concerning:

- *number of meals of freshwater fish/wildlife consumed in last 12 months; species (fish and wildlife) and location of catch (for fish only); source of fish.*
  In order to improve recall, the questions were asked for each season. While fish size is also important it was felt that the responses would not be reliable so a question on fish size was not included.

- *meal size for freshwater fish.*
  Plastic models of fish fillets, fillet pieces and steaks, similar to models that have been used previously in the Nutrition Canada survey, were developed to assess meal size. The methods used for determining meal size are described in more detail in Appendix 4.

- *historical freshwater fish and wildlife consumption.*
  The questionnaire asked how many years the respondent had been eating freshwater fish or wildlife, when they stopped eating freshwater fish or wildlife (if appropriate) and whether (and how) their freshwater fish and wildlife consumption habits had changed.

- *preparation and cooking methods used for freshwater fish.*
  Questions were included to describe Ontario angler practices and get an indication of whether anglers are following the recommended preparation and cooking methods provided in the "Guide to Eating Ontario Sport Fish".

- *knowledge and use of "Guide to Eating Ontario Sport Fish".*
  Since this guide contains information on fish consumption and preparation, it was useful to ask anglers about their awareness and use of the guide.

- *confounders, effect modifiers and identifying information.*
  These questions included occupation, age, gender, address, occupational and home exposure to environmental contaminants, education, income, number of children, breast-feeding history, health history, medication use, recent weight gain/loss, smoking habits, alcohol consumption, meals of saltwater fish and seafood, and recent (within 72 hours) consumption of alcohol, fish or wildlife.

The survey instrument was available in both French and English. It was pre-tested using three focus groups: Cornwall (English & French) and Mississauga (English only). Each focus group consisted of 5-6 anglers who consumed fish from the local area. Comments were also provided by two executive club members of the Credit River Anglers Association (Mississauga). As a result of the pre-test the phrasing of some questions was modified slightly, the layout of some questions was improved to reduce recording errors (particularly the large matrices in Questions 9 and 10) and instructions in the interviewers' training guide were improved.

6.4.3 Biological sampling

Blood, hair and urine samples were collected for contaminant residue analysis, clinical chemistry and biomarker analysis. The parameters measured are listed in Table 6-5. All contaminant residue analysis was done on individual samples except for co-planar PCBs, dioxin and furan quantification which was done on pooled samples.

Protocols for collection, sampling, preservation, and shipping of the samples are provided in Appendix 6, as well as analytical methodology, QA/QC methods and reference values, where available. Participants were asked not to consume fish for 72 hours prior to sampling.

The following samples were collected:

Blood

A total of 47 mL of blood was taken in 5 vacutainers according to the blood collection protocol given in Appendix 6. The samples were for the following purposes:

1) organochlorine pesticide/PCB scan (plasma)	10mL
2) sample for pooling for coplanar PCBs, dioxins, furans (plasma)	10mL
3) clinical chemistry and lipid profile (serum)	10mL
4) metals - Hg, Cd, Pb (whole blood)	7mL
5) chromosomal aberrations (whole blood)	10mL
TOTAL VOLUME	47mL

Urine

Participants were asked to collect their first morning void in a container provided previously, according to the protocol in Appendix 6. This was analyzed for:

1) Urinary porphyrins	min. 10mL
2) Urinary d-glucaric acid & urinary cotinine	min 10mL
TOTAL VOLUME	20mL

Hair

A bundle of scalp hairs, roughly 5 mm in diameter at the scalp, was collected from the back of the head for mercury analysis (Appendix 6). The hair sample was divided into centimetre sections at the laboratory.

Table 6-5
Measurements in Biological Samples
(and detection limits)

Organochlorine pesticides/industrial chemicals ***(in blood plasma)***

Parameter	Detection limit (µg/L)
p,p'-DDE	0.03
p,p'-DDT	0.03
dieldrin	0.10
β-hexachlorocyclohexane (β-HCH)	0.03
α-chlordane	0.02
γ-chlordane	0.02
oxychlordane	0.02
transnonachlor	0.02
heptachlor epoxide	0.10
mirex	0.02
hexachlorobenzene	0.02
Total PCBs as Ar1260	0.20
14 selected PCB congeners (28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187)	0.02

coplanar PCBs, dioxins and furans *(in pooled samples - currently underway)*

Metals (detection limits in brackets)

in whole blood

cadmium (0.2µg/L), lead (0.5µg/dL), mercury (total (2µg/L), (inorganic (2µg/L))

in hair

mercury (total (0.5µg/g), inorganic (0.5µg/g)) in 1st, 2nd, 3rd, 5th and 7th cm sections, where possible

Clinical Chemistry ***(in serum)***

Lipid profile	*Liver function enzymes*	*Thyroid hormones*
triglycerides	alanine aminotransferase	T3, T4
total cholesterol	alkaline phosphatase	TSH
free cholesterol	aspartate aminotransferase	
phospholipids	gamma glutamyl transferase	
	albumin	

Sub-clinical effect markers

Porphyrins in urine - measure of alterations of heme synthesis pathway (D. Grant/M. Feeley/S. Perkins)

D-glucaric acid in urine - measure of liver enzyme induction (R. Poon/M. Feeley/D. Villeneuve)

Chromosomal aberrations (blood lymphocytes) - measure of genetic damage induced by exposure to mutagenic agents (D. Blakey) *(currently underway)*

Confounding exposure marker

Cotinine in urine - cotinine, a metabolite of nicotine, is a marker for current cigarette or nicotine exposure.

6.4.3.1 Contaminant residue analysis

Organochlorine pesticides and PCBs

Blood plasma from each participant was analyzed for 11 organochlorine pesticides, total PCBs (as Aroclor 1260 and as the sum of the 14 congeners) and 14 individual PCB congeners listed in Table 6-5, by the Centre de Toxicologie du Québec. This was done using gas chromatography with electron capture detection, as described in Appendix 6. Aroclor 1260 was quantified using the peaks of congeners 138 and 153. Spiked samples were prepared and included randomly with shipments to the laboratory, throughout the study. Details of the methods used in preparing the spiked samples and the results of the analyses are provided in Appendix 6. A report on quality control was prepared by the laboratory (Appendix 6). Detection limits have been provided in Table 6-5. In a few cases, detection limits on individual samples were slightly higher due to insufficient sample volume. These detection limits were noted for each sample.

Dioxins, dibenzofurans and coplanar PCBs

Fourteen pooled serum samples were analyzed for polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and coplanar PCBs (congeners 77, 126 and 169), using high-resolution gas chromatography/mass spectrometry by Jake Ryan, Foods Directorate, Health Canada (methods described in Ryan et al 1994 and Appendix 6).

The total 'dioxin toxic equivalents' (TEQs) were calculated for each pooled sample. International 'toxicity equivalency factors' (TEFs) have been developed for PCDDs and PCDFs (Kutz et al 1990) and for PCBs (Ahlborg et al 1994). The TEFs range from 0.001 to 1; they quantify the toxicity of a specific congener relative to the most toxic dioxin congener, 2,3,7,8-TCDD, which has a TEF of 1. The total 'dioxin toxicity equivalents' of a mixture is calculated by multiplying the measured amount of each congener by its toxic equivalency factor (TEF) and summing these for all congeners.

The pooled samples were created by combining 10ml plasma samples from 10 individuals randomly selected from 12 (2x3x2) groups which were defined using the following variables:

1) Community - two categories: Cornwall and Mississauga.
2) Age - 3 categories (using the 33rd and 66th percentiles): <38 years, 38-50 years, >50 years.
3) Current Great Lakes fish consumption status - 2 categories: eater and non-eater.

For Cornwall fish-eaters there were sufficient numbers of participants in two age cateogries to also stratify by Aroclor 1260 (PCB) lipid adjusted plasma levels (using 2 categories (lower, higher) based on 50th percentiles: ≤486.28µg/kg, >486.28µg/kg).

The groups are identified below:

Group	**Community**	**Age**	**Current Great Lakes fish eater**	**Lipid weight based Ar1260 (µg/kg) (lower = below 50th %'ile, higher = above 50th %'ile)**
1	Cornwall	<38 years	Yes	Lower
2	Cornwall	<38 years	Yes	Higher
3	Cornwall	38-50 years	Yes	Lower
4	Cornwall	38-50 years	Yes	Higher
5	Cornwall	>50 years	Yes	Higher
6	Cornwall	<38 years	No	N/A
7	Cornwall	38-50 years	No	N/A
8	Cornwall	>50 years	No	N/A
9	Mississauga	<38 years	Yes	N/A
10	Mississauga	38-50 years	Yes	N/A
11	Mississauga	>50 years	Yes	N/A
12	Mississauga	<38 years	No	N/A
13	Mississauga	38-50 years	No	N/A
14	Mississauga	>50 years	No	N/A

Metals

Total and inorganic mercury, lead and cadmium were analyzed in whole blood by the Occupational Health Unit, Medical Services Branch, Health Canada. All metals were quantified using graphite furnace atomic absorption spectrometry with Zeeman background correction (see Appendix 6). Detection limits are provided in Table 6-5.

Hair samples were also analyzed for total and inorganic mercury by Medical Services Branch. Accumulation in hair is one of the routes of mercury elimination from the body. It is estimated that hair grows at a rate of 1 cm/month so analysis of successive segments of the hair can give a historical record of mercury exposure. The analysis was done on the 1st, 2nd, 3rd, 5th, and 7th, centimeter sections (where possible), giving an estimate of exposure during the previous 7 months.

6.4.3.2 Clinical chemistry and lipid profiles

Lipid profiles

A serum lipid profile was done in order to present and analyze the organochlorine data on a lipid basis. Data indicate that in humans, serum PCB levels are related to serum lipid levels (Brown & Lawton 1984). The lipid profile was carried out by the Ottawa Civic Hospital Laboratory using the serum clinical chemistry sample. Total lipids were estimated using the method described by (Phillips et al 1989). This method defines total lipids as a function of total cholesterol, free cholesterol, triglycerides and phospholipids, according to the following equation (all parameters in g/L):

$$\text{Total lipids} = 1.677\,(\text{Total cholesterol} - \text{Free cholesterol}) + \text{Free cholesterol} + \text{Triglycerides} + \text{Phospholipids} \quad (\text{Equation 1})$$

Plasma organochlorine contaminant levels were calculated on a lipid basis (μg/kg). Although it would have been preferable to have the lipid profiles done on the plasma samples, rather than serum samples, it has been reported that the difference in serum and plasma total cholesterol (when using EDTA anticoagulant) is in the 3% range (Cooper et al 1988). It has been assumed then that the serum lipid profiles are sufficient estimates of the plasma lipid levels for the purpose of adjustment of the organochlorine measurements.

Liver function measures

Circulating blood levels of alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase and gamma glutamyl transferase provided an indication of possible liver cell damage (Arias et al 1988). These parameters were measured in the serum sample by the Ottawa Civic Hospital.

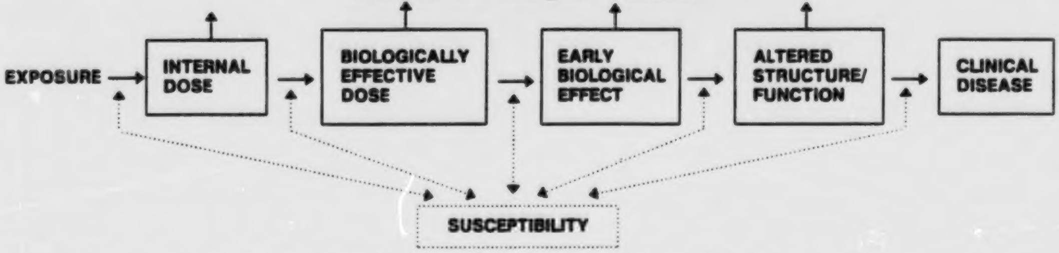
Thyroid hormones

Thyroid hormones (T_3, T_4, TSH) were measured in serum by the Ottawa Civic Hospital. Thyroid function is one of the biochemical markers that has been proposed as a bioindicator of persistent organochlorine contamination (Fox 1994) based on considerable evidence among wildlife (Environment Canada et al 1991). Routine clinical tests of thyroid function were abnormal in a larger percentage of polybrominated biphenyl workers than controls (Bahn et al 1980). The tests selected are among those regarded as effective for initial screening of thyroid dysfunction (Helfand and Crapo 1990).

6.4.3.3 Other sub-clinical effect and exposure markers

One of the study's secondary objectives was to examine the feasibility of using selected biomarkers in epidemiological studies of exposure to environmental contaminants. Depending on their degree of specificity and sensitivity, biomarkers can provide information on exposure, effects at the cellular or biochemical level, and susceptibility of individuals and populations to contaminant-related injury (Figure 6-4). The resulting information can be useful, in turn, in the identification of the populations at risk of subclinical injury.

Figure 6-4
Classes of biological markers



Source: Subcommittee on Reproductive and Neurodevelopmental Toxicology, 1989, p.17

Urinary porphyrins

Urinary porphyrins analyses were carried out by the Ottawa Civic Hospital in co-operation with Mark Feeley, Health Canada (laboratory methods are described in appendix 6). The liver is the major site of heme biosynthesis. Porphyrins and porphyrinogens are intermediates in the heme biosynthetic pathway. Inhibition of heme biosynthetic pathway enzymes can lead to an accumulation of these heme precursors, a condition known as porphyria. Changed liver porphyrin patterns associated with persistent organochlorine contamination have been extensively documented among gulls and other wildlife in the Great Lakes basin (Fox et al 1988). Urinary porphyrin profiles may also be indicative of heavy metal accumulation and nephrotoxicity. Liver disease may affect urinary porphyrin levels.

Urinary D-glucaric acid (UDGA)

D-glucaric acid was measured in urine by Dr. Raymond Poon, Health Canada using methods described in Poon et al (1993) (see appendix 6). D-glucaric acid is the most commonly used endpoint for measurement of D-glucuronic acid pathway activity in the human liver. It is a normal component of human urine. It has been demonstrated that exposure to known hepatic enzyme inducers, such as certain organochlorine compounds present in Great Lakes fish, results in significant increases in UDGA (Hunter et al 1972, Notten & Henderson 1977, Strik 1981). Preliminary studies demonstrate slight interindividual differences in UDGA levels in unexposed individuals (Poon et al 1993) thus providing a potentially useful biomarker for indirect assessment of hepatic enzyme activity. The following conditions/medications may affect the analytical results: (a) kidney or liver disease (b) pregnancy (c) progesterone or estrogen medications (oral contraceptives or hormone therapy) (d) phenobarbital medications.

Chromosomal aberrations

Selected chromosomal aberrations are being measured in cultured blood samples by Dr. David Blakey, Environmental Health Directorate, Health Canada (see Appendix 6). The measurement of chromosomal aberrations can be an indication of genetic damage induced by exposure to a variety of environmental pollutants. Although there are few direct correlations between the incidence of induced chromosomal aberrations in an individual and any specific health effect, a higher incidence of aberrations at a population level can provide evidence that exposure to chromosome-damaging agents has occurred. Exposure to other factors such as chemo-radiation therapy, ethylene oxide, gasoline (benzene), and coal tar (pitch) may also affect chromosomal aberration rates.

All samples were cultured shortly after sampling and stored until the questionnaire data were available. Structural chromosomal abberations are being examined in first division cells stained with Giemsa from selected samples from fisheaters, and suitable control samples matched by smoking status. In addition some samples will also be analysed for chromosomal translocations, using fluorescence *in situ* hydridization. Translocations are more stable then the first division aberrations detected using Giemsa staining alone, and are believed to accumulate during the life of an individual. Specific translocations have been associated with certain cancers and heritable genetic disorders.

Biomarker of cigarette consumption - urinary cotinine

Urinary cotinine was measured using a commonly available ELISA assay (SEREX) by Dr. R. Poon, Environmental Health Directorate Health Canada (see Appendix 6). Urinary cotinine is a metabolic product of nicotine metabolism. It is a promising biomarker to confirm or quantify smoking status (Wall et al 1988; Willers et al 1992, Cummings et al 1990, Sepkovic & Haley 1985). It has been used in a number of public health studies to quantify exposure to cigarette smoke among active, passive and non-smokers (Jarvis et al 1987, Vine et al 1993). Non-detectable levels were replaced by 4 ng/mL (½ the detection limit) for data analysis.

6.5 Stage 2 - Data management and analysis

All data management and analysis was done on a SUN SPARC workstation using SAS/UNIX software. Considerable data checking with original questionnaires was required. The extensive nature of individual reporting functions required considerable data management to ensure accuracy and appropriate interpretation. Some sets of data such as meal size required extensive additional work (see Appendix 4). At the aggregate level, all variables went through stages of data description, summary statistics, checks for normality, transformations as required, and synthesis into new variables when appropriate.

Different groupings of participants based on their sport fish consumption levels have been used for different analyses (see Table 6-6). Any participant who ate sport fish either from the 'Great Lakes' or from 'other lakes/rivers' was classified as a sport fish 'eater'. Any person who ate sport fish from any Great Lakes location[1] was also classified as a 'Great Lakes' fish eater. As well, any person who ate sport fish from any 'other lakes/river' location was classified as an 'other lakes' fish eater. For example, if a participant ate only sport fish from 'other lakes/rivers' they would be classified as a 'sport fish' eater but a 'Great Lakes fish' non-eater.

Table 6-6
Categories of fish consumers

Categories	Definition
'Sport' fish eaters vs non-eaters	'Sport' fish eater is a participant who ate at least one meal of sport fish from inland lakes/rivers or Great Lakes in the last 12 months.
'Great Lakes' fish eaters vs non-eaters	'Great Lakes' fish eater is a participant who ate at least one meal of 'Great Lakes' fish from the Great Lakes in the last 12 months.
'Other lakes' fish eaters vs non-eaters	'Other lakes' fish eater is a participant who ate at least one meal of fish from inland lakes/rivers (non Great Lakes) in the last 12 months.

Attempts have been made in Section 7 to clearly define which group has been used for particular analyses. Organochlorines are generally only of concern in Great Lakes fish and are a primary focus of this report. Hence, the analyses of the organochlorine data, potential confounders and biomarkers have focused on comparisons of Great Lakes fish eaters vs non-eaters. Similarly, data on cleaning methods, parts of fish eaten and cooking methods have been presented for Great Lakes eaters only, since these practices only affect organochlorines, not mercury levels. The analysis of mercury, cadmium and lead have been carried out on sport fish eaters vs non-eaters, because these contaminants can be in all sport fish.

---

[1] The Great Lakes locations included the rivers, such as the Credit River, which have species such as salmon and trout that spend most of their lives in Lake Ontario or another Great Lake. Also for Cornwall participants only, any perch purchased from a store or restaurant was considered to be Great Lakes sport fish. All other commercially purchased freshwater fish (eg. store or restaurant) were not considered in these analyses.

Geometric means have been provided when the data were log-normal. Some tables have been used to provide data for descriptive purposes only. In other cases, statistical tests of the significance of differences between means or percentages have been performed using χ^2 tests, t-tests or analysis of variance (when the data were considered to be normally distributed), or non-parametric tests as required (Wilcoxon rank sum or Kruskall-Wallis rank sum). The type of test used in an analysis has been provided in the text or as a footnote to the table. Generally p-values have been provided only when they were less than 0.05. Since this study is considered to be a hypothesis generating study, multiple comparisons have been made and the criterion for significance has been generally maintained at 0.05 - without correction.

For the contaminant data (organochlorines and metals), the percentages of samples with values below the detection limit have been presented. In any subsequent statistical analyses (such as correlations, regressions, means) non-detectable values have been replaced with a value equal to one-half the appropriate detection limit (see Table 6-5 for detection limits). Similarly, the total amount of PCBs was calculated by summing the 14 individual congeners after non-detectable values had been replaced with a value of 1/2 of the detection limit. Care must be taken however in interpreting statistical analyses of variables with large numbers of non-detectable levels.

Regression models have been presented for Aroclor1260, mercury, cadmium and lead (using the natural log transformed data). Potential exposure (eg. fish consumption) and confounder (eg. age, gender) variables were identified for each contaminant and included in a stepwise regression model to identify key independent variables for the models. Since this was considered a hypothesis generating analysis, a p-value of 0.10 was used for inclusion of main effect variables in the final model. Any two way interaction terms (of these selected main effect variables) which were significant at the $p=0.05$ significance level were also included in the final model. All variables involved in a two-way interaction term were included as main effect variables. P-values for Type I and Type III sums of squares (SS) have been provided. Type I sums of squares represent the incremental improvement in error sums of squares (SS) as each effect is added to the model (SAS Inst. Inc. 1988). Type III sums of squares are partial sums of squares (SAS Inst. Inc. 1988); the

Type III SS is equivalent to the Type I SS when the variable is the last one to be added to the model.

6.6 Dissemination of individual results

6.6.1 Results reporting

Preliminary clinical chemistry results - Jan/Feb 1993

Results from the clinical tests (cholesterol, liver enzymes and thyroid hormones) were provided by the laboratory as they were completed in early 1993. In consultation with the hospital clinical biochemist and the study's medical advisor, some test results were felt to be sufficiently out of the normal range to warrant immediate notification of the participant. The criteria for immediate notification for each parameter are given in Appendix 7.

If a participant had specified a physician, a letter was sent to their physician with the clinical test results, highlighting the abnormal value(s) and recommending follow-up, where necessary. The participant was sent a letter indicating that one of the clinical tests was out of the normal range and that they should contact their physician for possible follow-up.

If a participant did not have a physician, they were contacted by the study's medical advisor (by phone). In some cases, the participant did provide a physician's name at this point; in other cases the participant requested that the results be sent directly to them and they would take the results to a physician for follow-up.

Examples of the letters sent to the participants and physicians with preliminary clinical chemistry results are provided in Appendix 8.

Full reporting of individual results - July 7 1993

By March 1993, all analytical results were provided by the laboratories. The questionnaire data were available in early May 1993. On July 7 1993, letters were sent to each participant providing a summary and interpretation of their individual results. Letters were also sent to physicians with data sheets listing all of the participant's individual test results. Recommended follow-up was included in the physician's letter. When a participant did not report a physician, the data sheet (with all test results) was sent directly to the participant, with a covering letter which could be taken to a physician by the participant. Any clinical chemistry test results that had been provided earlier in Jan/Feb were included again in these packages.

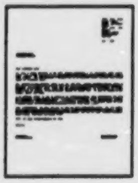
The information material prepared for the participants and physicians included 4 items: the covering letter and one-page summary sheet of results for the participant, and a covering letter and data sheet with test results for the physician (see Figure 6-5). For the participant, explanation and interpretation of each of the specific test results was included in the individualized summary sheet. For the physician, only unusual or 'abnormal' values were highlighted in the covering letter. These results were also flagged on the two-page data sheet. Examples of each of these items are provided in Appendix 9. Specific criteria were established for each parameter on which to base the interpretive text for the participant and physicians. For specific criteria and 'interpretive text' used in the participants summary sheet and the physician covering letter, see Appendix 7. As an example, blood lead guidelines have been established by Health Canada. Text was written for the participant and physician packages for people with blood lead levels below the guidelines; different text and recommendations were included in the package if the blood lead levels were above the guidelines.

In total, over 60 combinations of test results were found in the study group, so 60 different summary sheets and text sequences for physicians were required. As well, four different covering letters were written depending on whether the participant had received earlier results in Jan/Feb and whether or not they had a physician. Similar versions were required for the physician letters. Templates were prepared for each item using Wordperfect. Multiple merges were necessary to insert codes into the templates for each person which controlled which text sequences were included.

In addition to this material, a copy of a hand-out entitled "Having your catch and eating it too" was included in all participant and physician packages. A copy of this hand-out is included in Appendix 9.

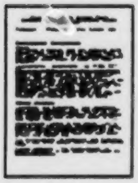
Figure 6-5 Materials sent to participants and physicians regarding individual results

To Participants



Covering letter (one page)

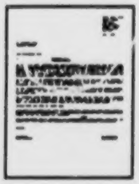
- described what information was being provided to them and their physician
- explained how to contact medical advisor or study coordinator to ask questions



Summary sheet (one page)

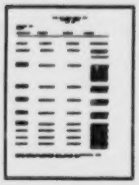
- attached to covering letter
- with summary and interpretation of results for each group of tests
- generally no raw data or test results included

To Physicians



Covering letter (one or two pages)

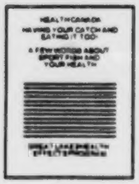
- brief study description
- highlighted results which were 'outside of reference ranges', 'above tolerable levels' etc. with brief interpretation (re. clinical significance) and advice on follow-up
- how to contact medical advisor
- if a participant did not specify a physician, the covering letter was prepared using 'Dear physician' salutation and was included with the data sheets (below) in the package sent to the participant (so they could take the material to a physician if they desired)



Data sheets (two pages)

- included all test results for a participant including reference ranges/tolerable levels for comparison and brief description of tests
- no interpretation of specific results was included in these sheets

To Participants and Physicians



'Having your catch and eating it too!'

- a hand-out on environmental contaminants in fish, and selecting and preparing fish for eating

6.6.2 Feedback on results reporting from physicians

Purpose

The labour intensiveness of our approach led us to want to evaluate its impact. We also had a responsibility to ensure that positive results in the "screening" process of the project were appropriately followed up without an excessive service burden on the health care system.

Methods and responses

Additional participant contact was an option but we were uneasy about making further demands on participants and were uncertain that they would know the specifics of some of the follow up. We therefore decided to contact the family physicians of those participants with contaminant levels above our guidelines (n=35) to obtain feedback on our information provision. We developed a questionnaire with open ended questions which the medical advisor used to interview physicians over the phone during the months of September to November, 1993.

The following questions were asked of each physician:

1. Was the information provided to the physician clear?
2. Did the physician discuss the information with the participant/patient?
3. What follow-up of test results occurred?
4. Is there any further information or activities for health professionals that physicians could suggest?

6.7 Dissemination of study results

Overall study results are being provided to the local public health units, the St. Lawrence Remedial Action Plan Team and Public Advisory Committee and the International Joint Commission. As well, the results will be distributed in the scientific community through conferences and published scientific papers. The authors are available for making presentations to interested groups.

The results of this study will be used in the planning of future health research and activities under the federal Great Lakes 2000 initiative.

7.0 RESULTS AND DISCUSSION

7.1 Participation rates

The overall rate of participation was 32.9% (232 out of 705 people contacted). A breakdown by community, fish eater status ('control' vs 'exposed') and recruitment phase is given in Table 7-1. Participation rates were generally higher in the 'exposed' (fish eaters) group than in the non-fish eater group ('controls') for each community.

Reasons for non-participation are listed in Table 7-2 for the 473 people who did not participate. In summary, of the 705 people sampled:

232 (33%)	participated in the study;
100 (14%)	could not be reached (moved, untraceable, deceased);
76 (11%)	did not participate due to logistical/scheduling problems, language problems or health problems;
124 (18%)	refused to participate with a specific reason noted and
173 (24%)	refused to participate but a specific reason was not noted by the field coordinator.

Of the refusals where a specific reason was noted by the field coordinator, about half were due to a dislike or fear of blood sampling/needles, etc. Many other refusals were due to a concern over the time or committment required without sufficient reason or benefit. In many cases the field coordinator reported spending a great deal of time discussing concerns with potential participants, but were not able to sufficiently alleviate concerns, especially surrounding the blood sampling.

The information gathered in the mail-out "Great Lakes Basin Anglers Survey" provides an opportunity to look at the characteristics of the participants versus the non-participants of the Exposure Assessment Study (see Table 7-3). Means or frequencies for each characteristic have been provided for the participants and non-participants, separately for the Cornwall mail-out respondents, Lancaster interviewees and Mississauga mail-out respondents. No significant differences were found in these characteristics in the Lancaster interviewees, but the sample size was small (22 participants and 23 non-participants).

In both the Cornwall and Mississauga mail-out samples, the calculated 'PCB index' (see Section 6.3.2) was higher for participants than non-participants which is another indication that fisheaters were more likely to agree to participate in the study than non-fisheaters. Consumption of wildfowl or game was much higher in the participants (55%) than

Table 7-1
Participation rates

	Sample	'Controls'			'Exposed'			Both		
		# Sampled	# Participants	Participation rate	# Sampled	# Participants	Participation rate	# Sampled	# Participants	Participation rate
First recruitment	Cornwall	101	16	16%	94	44	47%	195	60	31%
	Lancaster interviews	11	5	46%	18	6	33%	29	11	38%
	Mississauga	105	27	26%	112	40	36%	217	67	31%
	TOTAL	**217**	**48**	**22%**	**224**	**90**	**40%**	**441**	**138**	**31%**
Second recruitment	Cornwall	80	19	24%	80	40	50%	160	59	37%
	Lancaster interviews	8	5	63%	8	6	75%	16	11	69%
	Mississauga	0	0	--	88	24	27%	88	24	27%
	TOTAL	**88**	**24**	**27%**	**176**	**70**	**40%**	**264**	**94**	**36%**
Both recruitments	Cornwall	181	35	19%	174	84	48%	355	119	34%
	Lancaster interviews	19	10	53%	26	12	46%	45	22	49%
	Mississauga	105	27	26%	200	64	32%	305	91	30%
	TOTAL	**305**	**72**	**24%**	**400**	**160**	**40%**	**705**	**232**	**33%**

Note: 'Exposed' and 'control' groups, defined in Section 6.3.2, are based on information from the mail-out questionnaire.

Table 7-2
Reasons for non-participation
(number of people and percentages)

	Cornwall (n=259)	Mississauga (n=214)	Both (n=473)
Out of scope (moved, untraceable, deceased)	53 (20%)	47 (22%) [1]	100 (21%)
Language barrier	4 (2%)	5 (2%)	9 (2%)
Health			
• medical screening (fainting history, blood thinning medication)	3 (1%)	4 (2%)	7 (1%)
• other health reasons (illness, medical treatment, pregnancy)	18 (7%)	10 (5%)	28 (6%)
Logistic/scheduling problems			
• not possible during time period, cancellations	11 (4%)	13 (6%)	24 (5%)
• could not get blood	3 (1%)	5 (2%)	8 (2%)
Refusals			
• dislike/afraid of needles	23 (9%)	36 (17%)	59 (12%)
• study gone far enough/too involved/done enough/too busy/a lot of bother and trouble/spouse not keen/no point in study/does not want to know if something wrong	31 (12%)	34 (16%)	65 (14%)
• unspecified/multiple reasons	113 (44%)	60 (28%)	173 (37%)

[1] Percentages are percentages of records in which a specific reason for non-participation was recorded (not including "unspecified/multiple reason" category).

Table 7-3
Comparison of participants and non-participants[1,2]

Variable	Cornwall mailout		Lancaster interviews		Mississauga mailout	
	Participants (n=119)	Non-participants (n=236)	Participants (n=22)	Non-participants (n=23)	Participants (n=91)	Non-Participants (n=214)
Age[3] (mean)	43.2	41.6	49.1	52.4	42.1	43.3
% female	35%	53% (p=0.002)	41%	35%	35%	51% (p=0.011)
Education						
Grade 1-8	7%	17%	9%	13%	8%	8%
Grade 9-11	37%	31%	14%	30%	19%	23%
Completed high school	30%	30%	32%	39%	26%	24%
Some college or university	6%	5%	4%	9%	10%	12%
Completed college or university	19%	17%	41%	9%	36%	33%
Missing	1%	1%	0%	0%	1%	0%
Income						
<$30000	34%	49%	32%	52%	12%	20%
$30K-$59.9K	48%	31%	36%	17%	52%	41%
$>=$60K	8%	7%	14%	4%	33%	33%
Not given	9%	13% (p=0.008)	18%	26%	3%	7%
'PCB index' (mean) (µg/yr)	729.5	455.2 (p=0.03)	587.9	1016.8	619.5	540.3
% Wildfowl / game consumers	55%	27% (p<0.001)	27%	43%	33%	31%
% smokers	34%	36%	36%	35%	38%	30%
Drinks per week (mean)	6.3	3.9 (p=0.01)	4.4	12.2	7.2	5.3 (p=0.05)
Occupational solvent exposure						
Never	19%	25%	41%	52%	13%	32%
Occasionally	56%	53%	27%	35%	69%	52%
Often	16%	15%	32%	13%	15%	14%
Not given	8%	6%	0%	0%	2%	2% (p=0.007)
Occupational resin/isocyanate exposure						
Never	64%	69%	86%	83%	68%	80%
Occasionally	22%	16%	14%	17%	21%	13%
Often	3%	3%	0%	0%	3%	1%
Not given	11%	13%	0%	0%	8%	6%
Occupational pesticide exposure						
Never	37%	48%	73%	65%	40%	52%
Occasionally	49%	39%	27%	35%	54%	44%
Often	3%	2%	0%	0%	2%	1%
Not given	11%	10%	0%	0%	4%	3%

[1] All data are from mail-out/interview questionnaire: Great Lakes Basin Anglers Survey.

[2] P-values corresponding to t- (for means) or $\chi 2$ (for frequencies) tests of differences (between participants and non-participants) have been provided when less than 0.05.

[3] Age is age as of Jan 1/91

non-participants (27%, $p<0.001$) in Cornwall only. In Mississauga, wildfowl/game consumption was very similar in the two groups (33% vs. 31%).

The mean age was not significantly different between participants and non-participants in any of the three samples. However in both the Cornwall and Mississauga samples, females were less likely to participate than males (35% vs 65% in both samples). Education distributions were not different between the non-participants and participants. The income distribution was slightly higher in the participants than non-participants in both Cornwall and Mississauga, however this was only statistically significant in Cornwall.

Participants and non-participants did not differ significantly in smoking frequency, however in both Cornwall and Mississauga, the number of drinks per week were significantly higher in the participants than non-participants. In Mississauga, self-reported home or occupational exposure to solvents was significantly higher in participants than non-participants (84% vs 66% 'occasionally or often', $p=0.007$). Self-reported home or occupational exposures to resins/isocyanate and pesticides were not significantly different between participants and non-participants in either Cornwall or Missisauga.

7.2 Participant demographic characteristics

The focus will now be on the 232 people who participated in the biological sampling (stage 2).

Education and income levels were higher in the Lancaster and Mississauga samples than in Cornwall (see Table 7-3). The mean ages for each gender are provided for each sample in Table 7-4. Mean ages for men and women were similar as were mean ages in Cornwall and Mississauga. The Lancaster participants tended to be older, likely as a result of the recruitment method. The mean age was 44 for Cornwall (n = 119), 50 for the Lancaster interviewees (n = 22) and 43 for Mississauga participants (n = 91). The age range was from 18 to > 60 years with a good distribution across strata for both sexes. The percentage of female participants was between 35-41% for each sample (37% overall).

Table 7-4
Number of participants and mean age (by sample and gender)

	Female		**Male**		**Both**	
	n	**mean age (std. dev.)**	**n**	**mean age (std. dev.)**	**n**	**mean age (std. dev.)**
Cornwall	45(38%)	44.0(10.7)	74(62%)	44.0(11.2)	119	44.0(11.0)
Lancaster Interviews	9(41%)	47.9(15.9)	13(59%)	51.5(10.9)	22	50.0(13.0)
Subtotal	**54(38%)**	**44.7(11.6)**	**87(62%)**	**45.1(11.4)**	**141**	**44.9(11.5)**
Mississauga	32(35%)	43.8(10.3)	59(65%)	42.3(11.1)	91	42.9(10.8)
Total	**86(37%)**	**44.3(11.1)**	**146(63%)**	**44.0(11.3)**	**232**	**44.1(11.2)**

7.3 Sport fish and wildlife consumption

Current sport fish consumption

Freshwater fish consumption patterns are complicated by the diversity of participants activities. The left hand side of Table 7-5 shows the breakdown of participants eating fish from different sources in the last 12 months by community (as described in Section 6.5). The right hand side of the table indicates the categories of sport fish eaters that were derived from the definitions and data on the left hand side. To run through the table for one community, Cornwall had 31 (26%) "other lakes" eaters, 96 (81%) Great Lakes eaters and 100 (84%) sport fish eaters. The similarity of the consumption patterns of Great Lakes fish between the Cornwall and Lancaster participants and lack of more than one "other lakes" eater in Lancaster, led us to combine the Cornwall and Lancaster results for the rest of the report.

Table 7-5
Sport fish consumption categories

Fish consumption category	Number and percentages[1] of participants				Derived categories		
	Cornwall	Lancaster interviews	Mississauga	Totals			
Consumed only Great Lakes fish in the last 12 months	69 (58%)	12 (55%)	15 (17%)	96		Great Lakes fish "eaters" (n=152)	Sport fish "eaters" (n=176)
Consumed both Great Lakes and non-Great Lakes fish in the last 12 months	27 (23%)	1 (5%)	28 (31%)	56	Other Lakes fish "eaters" (n=80)		
Consumed only non-Great Lakes fish in the last 12 months	4 (3%)	0 (0%)	20 (22%)	24		Great Lakes fish "non-eaters" (n=80)	
Consumed no sport-caught fish in the last 12 months	19 (16%)	9 (41%)	28 (31%)	56			Sport fish "non-eaters" (n=56)
Total number of participants	119 (100%)	22 (100%)	91 (100%)	232			

[1] Percentages are % of participants in each community sample.

Fish species and locations

Table 7-6 lists the fish species eaten over the last 12 months and Figure 7-1 gives the locations from which they came. Cornwall 'sport fish eaters' fished primarily from the St.

Table 7-6
Fish species eaten in last 12 months
(number and percentages of fish eaters eating each species)

	from Great Lakes location		from "Other lakes" location	
	Cornwall (n = 109)	Mississauga (n = 43)	Cornwall (n = 32)	Mississauga (n = 48)
Bass, Largemouth	25 (22.9)	5 (11.6)	10 (31.3)	22 (45.8)
Bass, Smallmouth	49 (45.0)	8 (18.6)	10 (31.3)	28 (58.3)
Brown Bullhead	13 (11.9)	0 (0.0)	5 (15.6)	0 (0.0)
Burbot, Ling	4 (3.7)	1 (2.3)	0 (0.0)	0 (0.0)
Channel Catfish	4 (3.7)	1 (2.3)	0 (0.0)	2 (4.2)
Panfish[1]	9 (8.3)	4 (9.3)	1 (3.1)	5 (10.4)
Perch, Yellow	99 (90.1)	8 (18.6)	5 (15.6)	13 (27.1)
Perch, White	2 (1.8)	1 (2.3)	0 (0.0)	5 (10.4)
Pike, Northern	21 (19.3)	10 (23.3)	9 (28.1)	15 (31.3)
Rainbow Smelt	0 (0.0)	4 (9.3)	1 (3.1)	4 (8.3)
Salmon[2]	20 (18.3)	22 (51.2)	0 (0.0)	0 (0.0)
Trout, Brook	2 (1.8)	5 (11.6)	6 (18.8)	5 (10.4)
Trout, Brown	5 (4.6)	3 (7.0)	1 (3.1)	2 (4.2)
Trout, Lake	14 (12.8)	6 (14.0)	5 (15.6)	6 (12.5)
Trout, Rainbow	12 (11.1)	21 (48.8)	4 (12.5)	7 (14.6)
Walleye (pickerel)	59 (54.1)	6 (14.0)	16 (50.0)	23 (47.9)
Whitefish	0 (0.0)	5 (11.6)	1 (3.1)	6 (12.5)
Other species	3 (2.7)	6 (14.0)	4 (12.5)	7 (14.6)

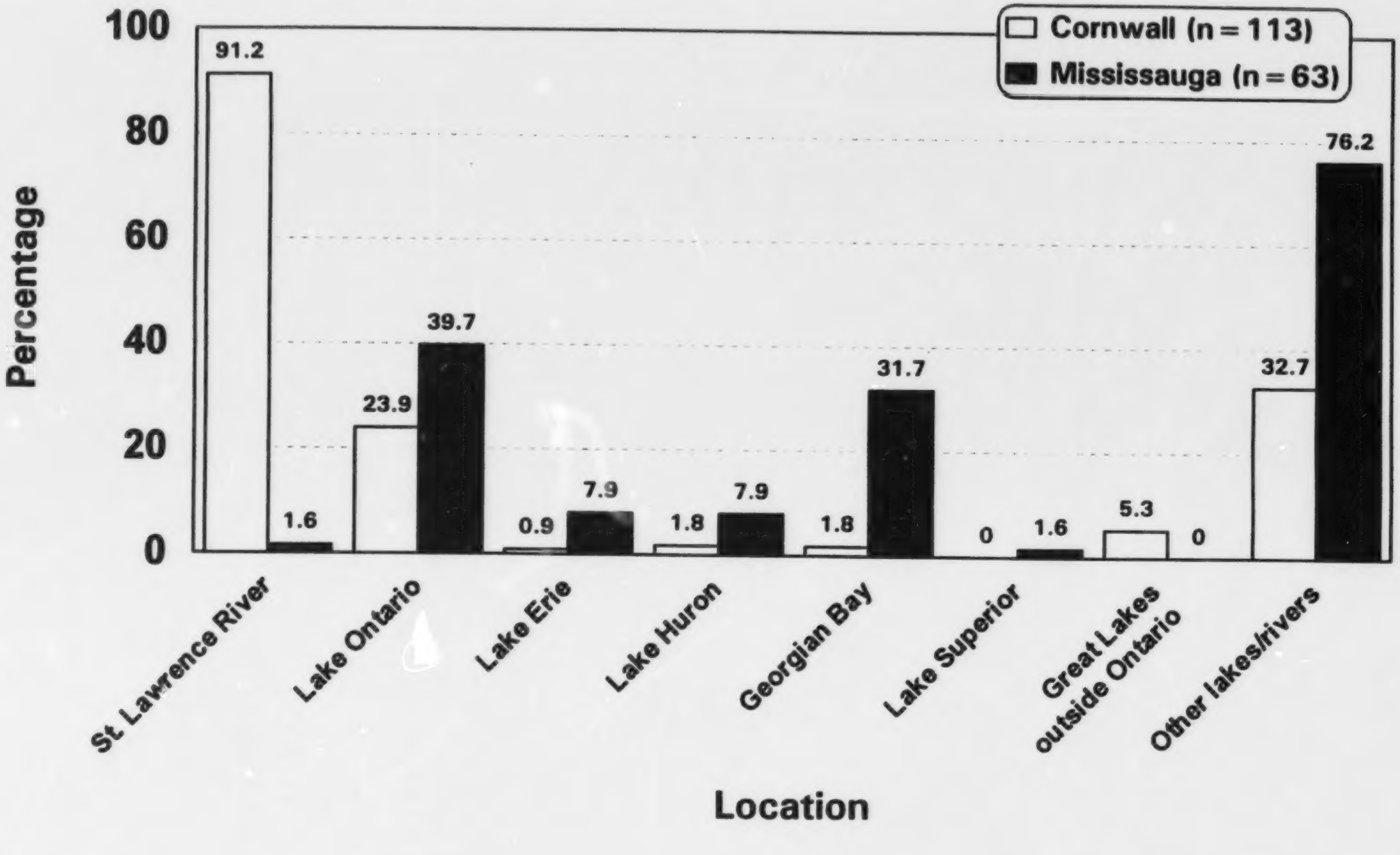
[1] 'Salmon' includes coho, chinook, pink salmon and "salmon-unknown species".

[2] 'Panfish' includes pumpkinseed, bluegill, rock bass, black crappie, white crappie and "panfish-unknown species".

Figure 7-1

Fishing Locations

Percentage of "sport fish" eaters eating fish from each location



Lawrence River (91%), 'other lakes/rivers' (outside the Great Lakes) (36%) and Lake Ontario (24%). Of those participants who ate fish from the Great Lakes, almost all ate yellow perch (90%) and over half ate walleye (pickerel).

Seventy-six percent of Mississauga 'sport fish eaters' ate fish from 'other lakes/rivers', followed by fish from Lake Ontario (40%). For the Mississauga participants who ate fish from the Great Lakes, salmon (51%) and rainbow trout (49%) were the most popular species. For the Mississauga participants who ate fish from 'other lakes/rivers', smallmouth bass was the most popular (50%), followed by walleye (48%) and largemouth bass (46%).

Meal size, frequency and total mass eaten

Meal sizes were estimated for each species eaten by each participant, using the methods described in Appendix 4. The mean, median and ranges of meal sizes for each species and community for all sport fish eaters are provided in Table 7-7, for the more frequently eaten species. Meal sizes vary considerably between species and community. For the most commonly eaten species (eaten by at least 10 people in a community) the median meal size ranged from 126.4-270.3 g; the mean meal size ranged from 166.1 - 547.6 g. (Meal size distributions are skewed to the right as indicated by the means being much larger than the medians; Kruskal-Wallis rank tests have been used to test for significant differences). For most species meal sizes were generally larger in Mississauga than in Cornwall, although the differences were only statistically significant for yellow perch ($p = 0.03$) and "salmon, unknown species" ($p = 0.03$).

For each sport fish eater, a mean mealsize was calculated by averaging the mealsizes for all species (eaten by that sport fish eater). The mean of these meal sizes was significantly higher for Mississauga sport fish eaters (mean 303.3 g), than for Cornwall sport fish eaters (mean 194.0 g) ($p = 0.005$, Table 7-8). However the average number of sport fish meals eaten in the last 12 months was far greater in Cornwall (37.7) than Mississauga (19.3) ($p = 0.0001$) so that the total kilograms of sport fish eaten in the last 12 months was similar in the 2 communities (7.79 kg in Cornwall vs 7.69 kg in Mississauga).

Looking at Great Lakes fish meals only, again the average meal size was significantly higher for Mississauga eaters (331.9 vs 186.1 g, $p = 0.0007$) but the mean number of Great Lakes fish meals was much higher in Cornwall (34.2 vs 10.9, $p = 0.0001$) (Table 7-8). Consequently the average total kilograms of Great Lakes fish eaten was higher in Cornwall than Mississauga (7.27 vs 4.50 kg, $p = 0.02$).

Table 7-7
Meal size by species
(grams)
(all sport fish eaters)

	Cornwall		Mississauga	
Species	**n**	**mean(std.dev.) median range**	**n**	**mean(std.dev.) median range**
Bass, largemouth	31	232.2 (96.8) 218.9 96.3-502.0	23	307.4 (328.5) 160.2 62.8-1429.0
Bass, smallmouth	53	232.3 (134.2) 192.5 55.7-875.5	31	234.7 (149.0) 192.5 27.9-640.7
Brown bullhead	14	346.3 (276.3) 270.3 109.5-1193.7	0	---
Perch, yellow	101	166.6 (158.3) 126.4 16.5-901.8	16	454.4 (568.4) 263.7 22.9-1961.2 (p=0.03)[1]
Salmon, chinook	6	364.8 (218.2) 377.5 137.8-573.4	13	237.6 (173.7) 200.1 88.1-758.5
Salmon, coho	7	309.7 (241.9) 215.0 60.6-573.4	10	547.6 (699.8) 218.6 88.1-2159.9
Salmon, unknown sp.	4	132.2 (57.8) 110.6 92.6-215.0	3	486.9 (288.2) 397.4 254.1-809.3 (p=0.03)[1]
Trout, lake	17	241.1 (308.4) 141.4 38.5-1360.8	10	238.7 (141.2) 200.0 70.7-513.2
Trout, rainbow	14	202.3 (163.7) 140.9 61.6-672.8	25	363.7 (468.6) 152.6 40.6-2023.9
Walleye	61	199.1 (130.9) 170.6 23.3-600.3	25	249.8 (230.1) 186.3 56.8-1220.7

[1] P-values from a Kruskal-Wallis test for differences between communities have been provided when $p<0.05$.

Table 7-8

Meal size, meal frequency and total mass eaten in last 12 months

arithmetic mean (standard deviation)/median/range)[1]

	Sport fish meals			Great Lakes fish meals			"Other Lakes" fish meals		
	Cornwall (n = 113[2])	Mississauga (n = 63)	Both (n = 176)	Cornwall (n = 109)	Mississauga (n = 43)	Both (n = 152)	Cornwall (n = 32)	Mississauga (n = 48)	Both (n = 80[2])
Mean mealsize (g)	194.0 (130.2) 168.0 16.5-737.6	303.3(266.3) 220.6 27.9-1567.6 (p = 0.005)[1]	233.4(197.1) 171.5 16.5-1567.6	186.1(120.1) 160.5 16.5-549.5	331.9(263.8) 225.2 22.9-989.2 (p = 0.0007)[1]	227.4(184.4) 175.2 16.5-989.2	244.1(289.5) 211.9 7.7-1680.9	291.1(284.8) 196.0 27.9-1567.6	272.7(285.8) 199.3 7.7-1680.9
# of meals	37.7(37.4) 27.0 1-173.5	19.3(24.6) 9 1-96 (p = 0.0001)[1]	31.1(34.5) 19.5 1-173.5	34.2(35.0) 24 1-173.5	10.9(14.2) 5.0 1-58.0 (p = 0.0001)[1]	27.6(32.3) 16 1-173.5	16.5(20.8) 6.5 1-97	15.6(22.3) 6.0 1-87	16.0(21.6) 6.5 1-97
Total mass (kg)	7.79(11.0) 3.96 0.05-64.6	7.69(15.8) 1.83 0.03-85.2	7.76(12.9) 3.41 0.03-85.2	7.27(11.0) 3.52 0.05-64.6	4.50(9.0) 1.28 0.02-51.6 (p = 0.02)[1]	6.48(10.5) 2.34 0.02-64.6	2.77(3.05) 1.52 0.02-12.3	6.06(13.4) 1.48 0.03-62.4	4.74(10.6) 1.48 0.02-62.4

[1] p-values from a Kruskal-Wallis test for differences between communities are provided when < 0.05.

[2] 1 person had a missing mealsize.

Sport fish consumption figures for this and other studies are presented for comparison in Table 7-9. Daily consumption estimates for all sport fish (eaters only) were 21.3 g/day in Cornwall and 21.1 g/day in Mississauga. These rates are similar to those found by Cox et al, 1987, 1990, 1993 for Ontario license holders, and higher than the rate (14.2 g/day) found in Lake Ontario derby anglers (Cox et al, 1990). Sport fish consumption rates in this study were higher than that found in Wisconsin fisheaters by Fiore et al (1989) (12.3g), similar to those of Ontario and New York State license holders, and Vietnamese women in Hamilton (Ontario), and lower than that found in Gulf of St. Lawrence Lower North Shore fishermen, and in an earlier study of Lake Michigan anglers and fisheaters (see references in Table 7-9).

Table 7-9
Mean sport fish consumption estimates in this and other studies
(eaters only)

Reference	Study group	n	meals/year	mealsize (grams)	g/day
This study	All sport fish meals				
	Cornwall	113	37.7	194.0	21.3
	Mississauga	63	19.3	303.3	21.1
	Great Lakes meals only				
	Cornwall	109	34.2	186.1	19.9
	Mississauga	43	10.9	331.9	12.3
Cox et al, 1987	"Ontario Guide to Eating Sport 1983	807		284	
Cox et al, 1990	Fish" respondents 1986	1337	30.6	295	24.7
Cox et al, 1990	1989	913	27.0	272	20.2
Cox et al, 1993	1992	494	30.0	276	22.5
Cox et al, 1990	Lake Ontario derby anglers 1989	603	21.1	246	14.2
Cole D. pers. commun., 1995	Vietnamese women, Hamilton, Ontario 1993	20	47.7	180	22.6
Dewailly et al, 1991	Gulf of St. Lawrence Lower North Shore fishermen and families 1990	195			75.0
Connelly et al, 1992	New York State fish license holders 1992	1030			27.4
Fiore et al, 1989	Wisconsin anglers (eaters only) 1984 (sport caught fish only)	192	19.8	227 (assumed by author)	12.3
Richardson & Currie 1993	Ontario Amerindians (estimated from Hg hair levels)	4327			16.2
Humphrey 1983[1]	Lake Michigan fisheaters 1973-74	96	60	284	39.9
	1980	96	39	227	26.2

[1] Data for Humphrey 1983 are medians.

Sport fish cleaning, parts eaten and cooking methods

The questionnaire used in this study asked for information on the way fish were cleaned, what parts were eaten and how the fish were cooked (Question 10). For each of these practices, a dichotomous variable was created (1 = usually or sometimes; 0 = never). The percentage of respondents using each method/practice or eating a particular part of the fish are presented for the most common fish species in Appendix 5.

The data were aggregated to create overall cleaning, parts eaten and cooking summary measures for each participant based on literature indicating practices that should result in greater or lower intake of contaminants (described in Figure 7-2). These summary measures were averaged across species for each participant. The mean measures (and standard deviations) are provided in Table 7-10 averaged for each community for Great Lakes fish eaters. The reported mean 'cleaning' measures were not significantly different in the two communities. In Cornwall the mean summary measure for 'parts eaten' was higher (3.81 vs 3.43, $p = 0.0001$, Kruskal Wallis test) and the mean summary measure for 'cooking' was lower (2.42 vs 2.98, $p = 0.0001$, Kruskal Wallis test) than in Mississauga, possibly reflecting different species eaten (i.e. more fried perch fillets).

Figure 7-2
Fish cleaning, parts eaten and cooking method summary measures
(from Question 10)

Fish cleaning methods
1. cut off and discard the belly meat
2. cut off and discard the fat from the back
3. cut off and discard the fat from the sides
4. remove the skin
5. puncture the skin

Summary "cleaning" measure:

number of methods used 'usually' or 'sometimes' (min = 0; max = 5)

Parts eaten
1. whole fish, less head, fins, tail, guts and bones
2. fillets
3. steaks
4. skin

Summary "parts eaten" measure:
base value = 3
if eat whole fish 'usually' or 'sometimes' then subtract 1
if eat skin 'usually' or 'sometimes' then subtract 1
if eat fillets 'usually' or 'sometimes' then add 1
if eat steaks 'usually' or 'sometimes' then add 1

Cooking methods
1. deep fat fry or pan fry it
2. re-use any of this fat
3. bake, broil, grill or BBQ it
4. poach, boil, microwave or steam it

Summary "cooking" measure:
base value = 3
if deep fat or pan fry 'usually' or 'sometimes' subtract 1
if re-use any of fat 'usually' or 'sometimes' subtract 1
if bake, broil, grill or BBQ it 'usually' or 'sometimes' add 1
if poach, boil, microwave or steam it 'usually' or 'sometimes' add 1

Note: for all 3 summary measures, a higher measure implies a possible reduction in organochlorine contaminant intake from fish.

Table 7-10
Cleaning, parts eaten and cooking summary measures
(for Great Lakes fish eaters)
(arithmetic mean (standard deviation))

	Cornwall (n = 109)	Mississauga (n = 43)
Cleaning	2.78 (1.09)	2.83 (1.25)
Parts eaten	3.81 (0.36)	3.43 (0.71)
Cooking	2.42 (0.59)	2.98 (0.73)

Historical sport fish consumption

Because of the long half lives of persistent organochlorines in the human body (estimated at up to 7 years), historical consumption may contribute considerably to present body burden. In Table 7-11 participants are categorized into those who have never eaten Great Lakes fish, those who ate Great Lakes fish at one time but have since stopped (former) and those who currently eat Great Lakes fish. Less than a quarter of participants had never eaten Great Lakes fish (56/232). Twenty-four (10%) had eaten Great Lakes fish for a mean of 23 years up until a mean of 7.1 years ago. Such participants could still have half the body burden of some organochlorines that they had when they stopped eating. Among eaters, subtracting their mean number of years eating (33) from their mean age (44) results in a starting age of catching and eating of 11, i.e., most anglers start their consumption in childhood and adolescence. Current age and the number of years eating sport fish were therefore included in subsequent modelling of the PCB (Ar1260) data.

Table 7-11
Historical fish consumption

Great Lakes fish eating status	Cornwall	Mississauga	Both
Never			
# participants	19 (14%)	37 (41%)	56 (24%)
Former			
# participants	13 (9%)	11 (12%)	24 (10%)
# years since eating Great Lakes fish (mean (std. dev.))	10.2 (10.7)	3.5 (4.8)	7.1 (9.0)
# years eating Great Lakes fish (mean (std. dev.))	26.0 (22.3)	19.4 (14.0)	23.0 (18.9)
Current			
# participants	109 (77%)	43 (47%)	152 (65%)
# years eating Great Lakes fish (mean (std. dev.))	33.8 (13.0)	31.3 (13.3)	33.1 (13.1)
Total number of participants	141	91	232

Sport fish 'eaters' were also asked if they had changed their consumption (amount, location or species) of freshwater fish in the last 10 years (see Table 7-12). In Cornwall 63 of 113 (56%) sport fish 'eaters' had changed their consumption, predominately in the *amount* of fish they consumed - 40 (35% of the 113 sport fish eaters) ate less fish now than 10 years ago and 21 (19%) ate more fish now. Fifteen (13%) sport fish eaters ate different *species* of fish and fifteen (13%) ate from different *locations* than 10 years ago (9 of the 15 reported that they no longer ate from certain locations in the St. Lawrence River).

In Mississauga 46% of the 63 sport fish eaters had changed their consumption in the last 10 years, again predominately in the *amount* of fish consumed - 17 (27%) ate less fish now and 8 (13%) ate more fish now than 10 years ago. Nine (14%) of sport fish eaters had changed the *species* and 9 (14%) ate fish from different *locations* than 10 years ago (8 of the 9 participants reported that they used to eat from Lake Ontario or the Credit River).

Table 7-12
Number of sport fish eaters reporting changes in amount eaten, species and location during last 10 years

	Cornwall (n = 113 sport fish eaters)	Mississauga (n = 63 sport fish eaters)
Changed consumption in last 10 years (amount, species or location)	63 (56%)	29 (46%)
Changed amount eaten		
Eat greater amount now than 10 years ago	21 (19%)	8 (13%)
Eat lesser amount now than 10 years ago	40 (35%)	17 (27%)
Changed species		
Eat different species now than 10 years ago	15 (13%)	9 (14%)
Changed location/sources		
No longer using some locations/sources for fish	15 (13%)	9 (14%)

Awareness and use of the "Guide to Eating Ontario Sport Fish"

Participants were asked about their awareness and use of the Guide to Eating Ontario Sport Fish (Ontario Ministry of Environment, 1991), which contains detailed advice on selecting fish for eating from Ontario lakes and rivers, including the Great Lakes.

Eighty-five percent of participants were aware of the guide, however only 28% of participants had a copy and followed the advice at least sometimes (Figure 7-3). Others knew about it but did not have a copy at the present time (30%), which would not exclude their having had a previous year's version, as in a New York survey of anglers (Connelly et al 1992). Twenty-seven percent of participants had a copy of the guide but 'never' followed the advice.

The percentage of participants who had a Guide and followed the advice at least sometimes was higher in Mississauga (34.6%) than Cornwall (23.9%) (see Figure 7-3). In Mississauga 33.3% of participants had a copy but never followed the advice, compared with 23.0% in Cornwall. A greater percentage of participants in Cornwall were aware of the Guide but did not have a copy (34.5% compared with 22.2% in Mississauga). A greater percentage of participants were unaware of the Guide in Cornwall (18.6%) than in Mississauga (9.5%).

Higher levels of education were found to be associated with awareness and use of the Guide, in both communities (Figure 7-4). The average income level (Figure 7-5) was higher for participants aware of the Guide compared with those not aware of the Guide in both communties, but higher income was not consistently related to having or following the Guide.

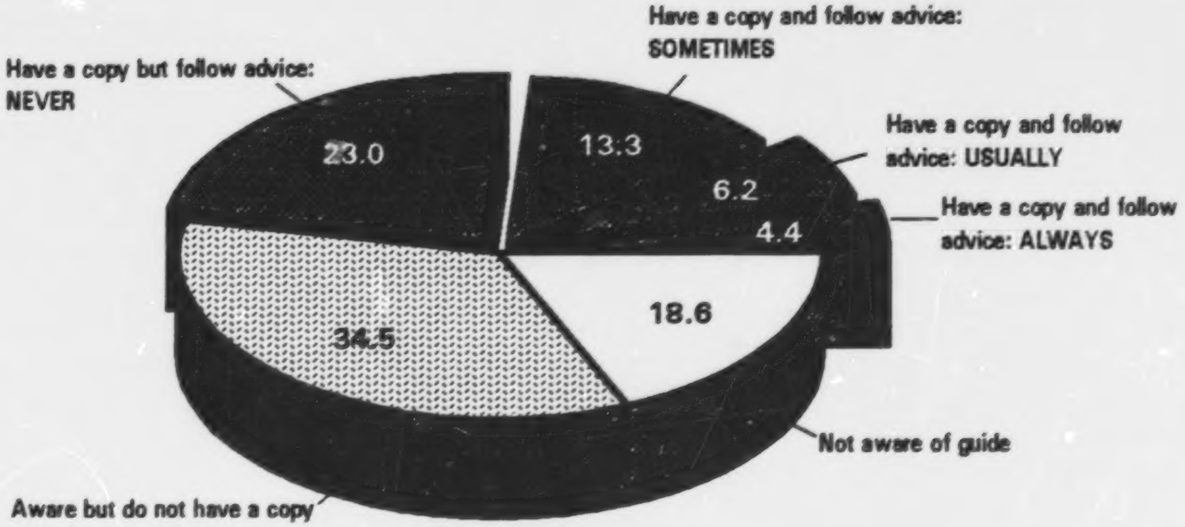
In Cornwall, the highest average 'cleaning' summary measure was in the group who 'had a copy and followed advice' (see Table 7-13; see Figure 7-2 for definitions of summary measures) and the lowest value was in the group who were 'not aware of the Guide'. However the differences were not statistically significant. The scores for 'parts eaten' were higher in the group "not aware of the Guide" (p=0.05 Kruskal Wallis test). The mean 'cooking' summary measures were not significantly different across groups. In Mississauga there were no significant differences in any of the measures across Guide use categories.

Figure 7-3

Awareness and Use of "Guide to Eating Ontario Sport Fish"

(percentage of "sport fish" eaters)

Cornwall (n = 113)



Mississauga (n = 63)

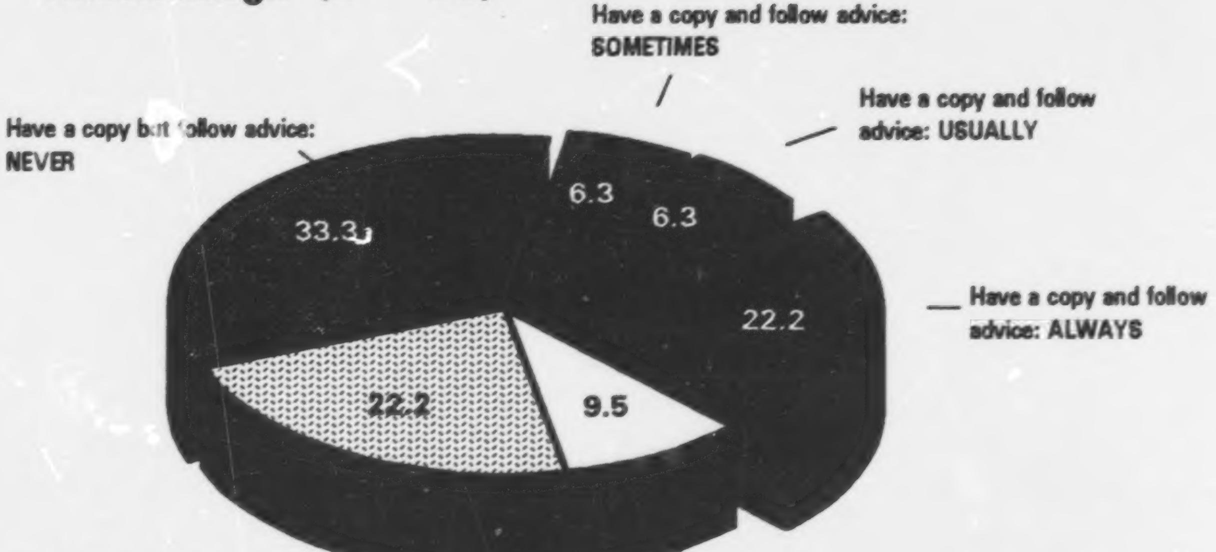
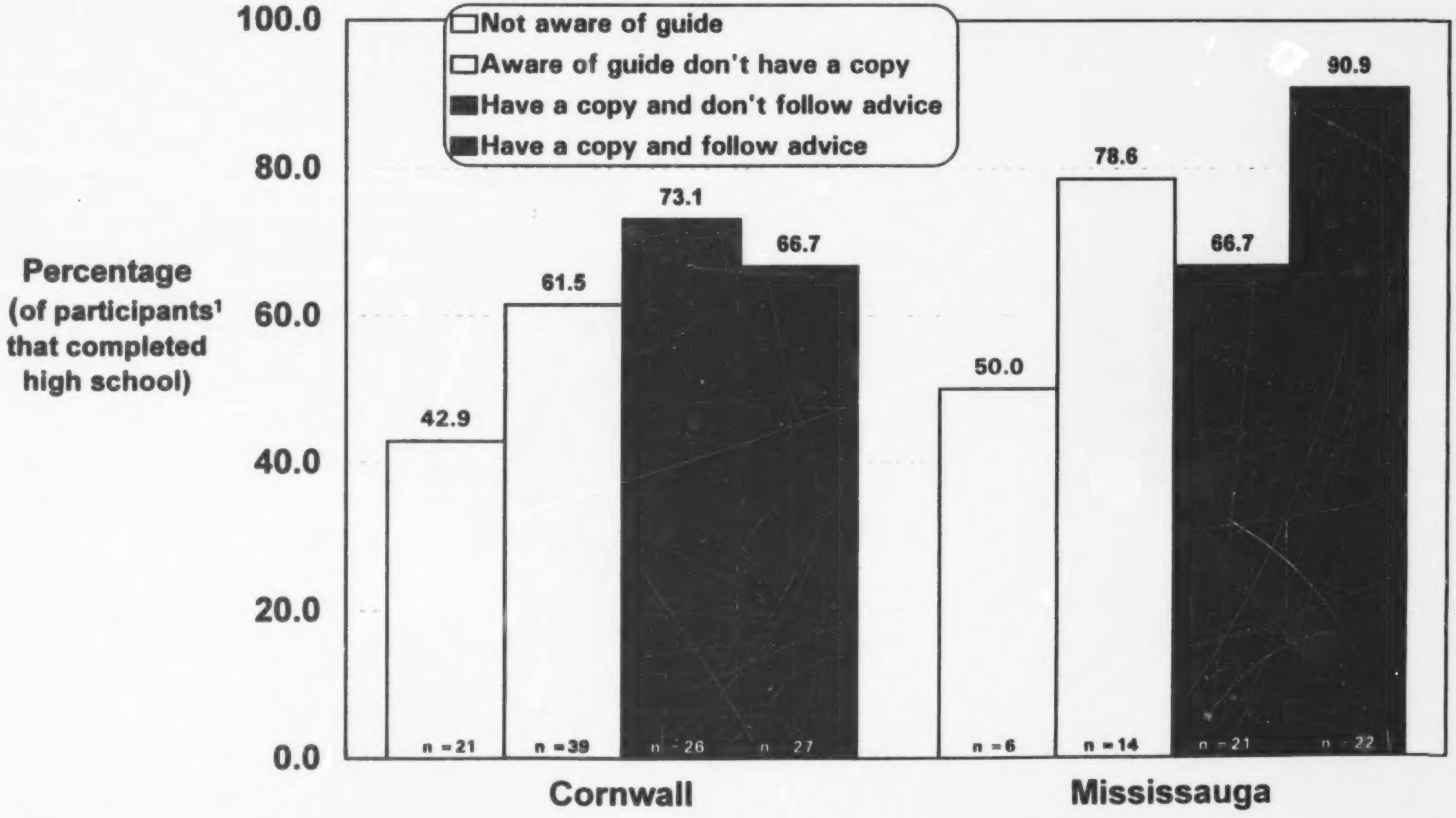


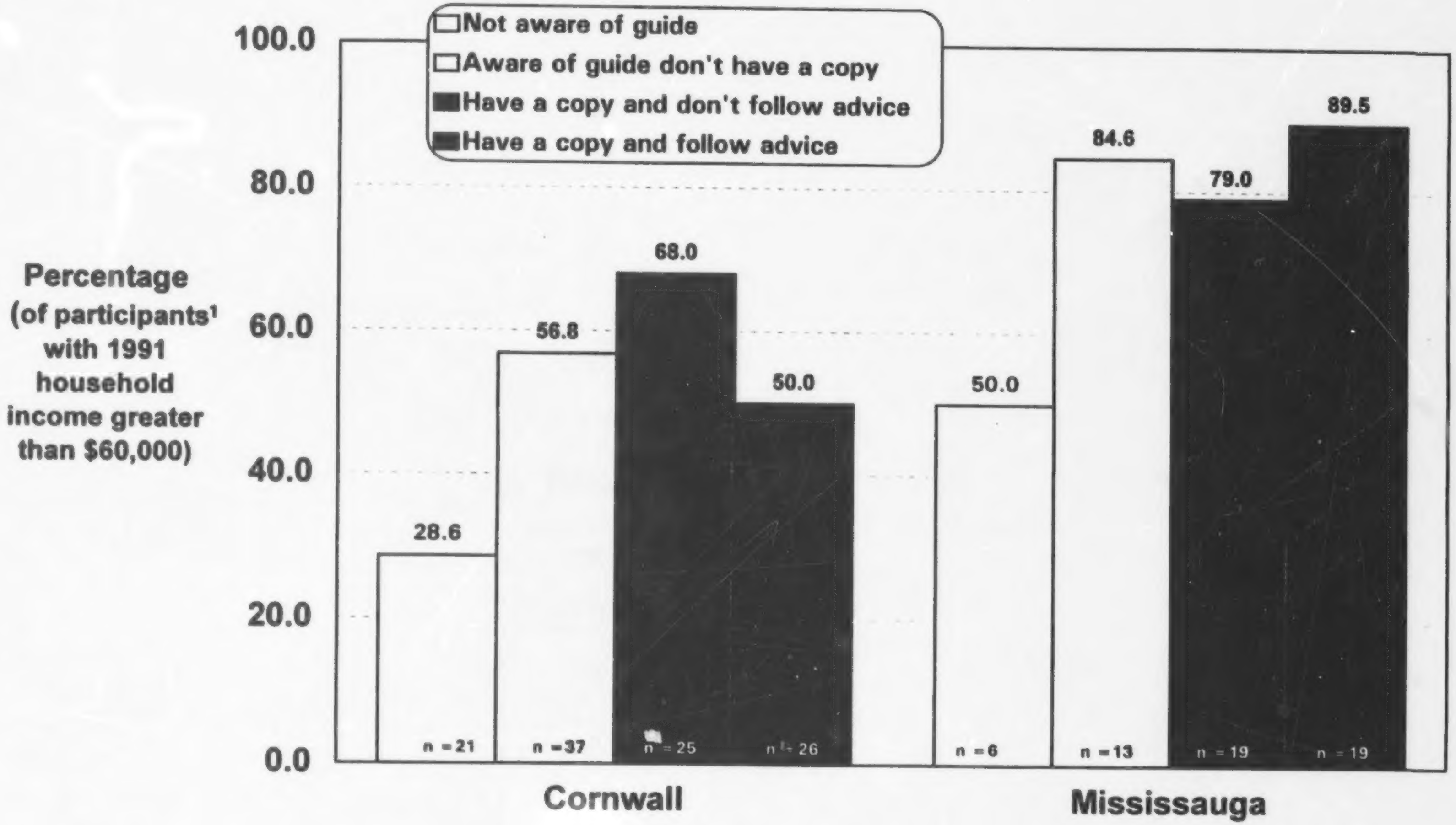
Figure 7-4
Awareness of guide and education level



---

[1] % is % of participants within each guide use category

Figure 7-5
Awareness of guide and income level



[1] % is % of participants within each guide use category

Table 7-13
Guide use and mean measures for cleaning, parts eaten and cooking (for Great Lakes fish eaters)

	Cornwall				**Mississauga**			
	n	**Cleaning**	**Parts eaten**	**Cooking**	**n**	**Cleaning**	**Parts eaten**	**Cooking**
Have a copy and follow	26	3.16	3.75	2.39	17	3.06	3.56	3.00
Have a copy and don't follow	25	2.68	3.74	2.48	12	2.53	3.45	3.10
Aware of Guide; don't have a copy	38	2.72	3.82	2.47	10	2.71	3.28	3.04
Not aware of Guide	20	2.50	3.98	2.27	4	3.08	3.21	2.40

Note: No significant differences across Guide user groups were found in either community for the 3 measures (using Analysis of Variance).

Wildlife consumption

Significantly more sport fish eaters also ate game or wildfowl, ducks or geese and moose and deer than non fish eaters in both communities (Table 7-14). A greater percentage of participants in Cornwall consumed wildlife in the last 12 months (53% vs 34% in Mississauga).

Thirty-three (33) Cornwall participants reported eating ducks or geese in the last 12 months. The following species were reported eaten (the number of participants is in partentheses): geese (26), mallard (17), black duck (10), teal (8), bluebill (scaup) (6), wood duck (3), whistlers (goldeneye) (1), unspecified duck speces (3). Only six participants in Mississauga reported eating ducks or geese in the last 12 months. The species were: mallards (5), wood duck (1) and unspecified duck species (1). The number of ducks/geese meals eaten in the last 12 months ranged from 1 to 34 with a median of 4 in Cornwall; in Mississauga the number of meals ranged from 1 to 8 with a median of 2.

The number of moose meals (n=57 eaters) ranged from 1 to 52 with a median of 3. The number of deer meals (n=67 eaters) ranged from 1 to 80 with a median of 4.

Local duck or geese consumption could contribute to environmental contaminant intake so duck/geese consumption was included, if statistically significant, in the models for PCBs (Ar1260), mercury and lead (presented in the following sections). Moose/deer consumption may contribute to cadmium intake so it was included in the statistical modelling for cadmium.

Table 7-14
Percentage of participants consuming wildlife and waterfowl
(in last 12 months)

	Cornwall		Mississauga		Both	
	Sport fish		Sport fish		Sport fish	
	"non-eaters" (n=28)	"eaters" (n=113)	"non-eaters" (n=28)	"eaters" (n=63)	"non-eaters" (n=56)	"eaters" (n=176)
Game or waterfowl	17.9	64.6 (p<0.001)[1]	7.1	46.0 (p<0.001)[1]	12.5	58.0 (p<0.001)[1]
Ducks or geese (meat or organs)	0.0	29.2 (p=0.001)[1]	0.0	9.5 (p=0.09)[1]	0.0	22.2 (p<0.001)[1]
Duck/geese liver or kidney	0.0	0.9	0.0	1.6	0.0	1.1
Duck/geese heart or gizzard	0.0	0.9	0.0	1.6	0.0	1.1
Moose or deer (meat or organs)	17.9	55.8 (p<0.0001)[1]	7.1	33.3 (p=0.008)[1]	12.5	47.7 (p<0.001)[1]
Moose	3.6	38.1 (p<0.001)[1]	3.6	23.8 (p=0.02)[1]	3.6	33.0 (p<0.001)[1]
Moose liver	0.0	1.8	0.0	0.0	0.0	1.1
Deer	14.3	46.9 (p=0.002)[1]	3.6	20.6 (p=0.04)[1]	8.9	37.5 (p<0.001)[1]
Deer liver	0.0	3.5	0.0	3.2	0.0	3.4
Moose/deer heart of other organs	0.0	4.4	0.0	1.6	0.0	3.4
Bullfrog	3.6	4.4	0.0	4.8	1.8	4.6

[1] P-value from a χ2 test

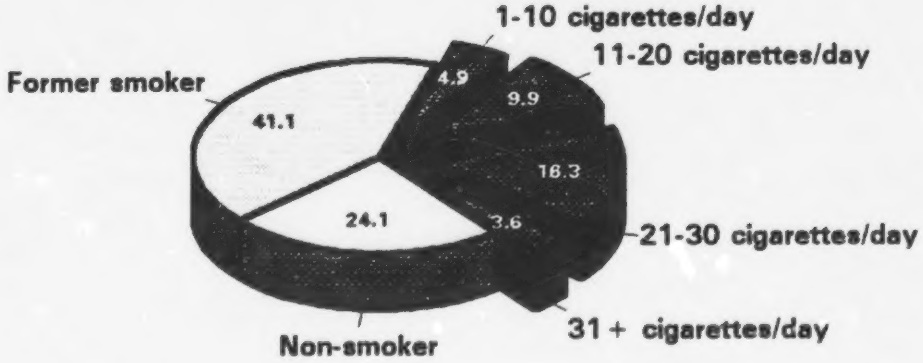
7.4 Other exposures and potential confounding variables

Smoking is an important factor in interpreting cadmium blood levels and liver function results. A detailed breakdown of smoking by community (Figure 7-6) shows that smokers in Cornwall tended to smoke more cigarettes/day (mean 21.3 cigs/day) than those in Mississauga (mean 18.6 cigs/day), although the difference was not significant ($p=0.26$, χ^2 test). Table 7-15 shows that Great Lakes fish 'eaters' are more likely to be current or former smokers than the 'non-eaters' (37% vs 31%) although the difference was not significant ($p=0.40$, χ^2 test).

In Cornwall, mean alcohol consumption was significantly lower among 'non- eaters' than 'eaters' (3.7 vs. 6.9 drinks/week; $p=0.023$,Wilcoxon rank sum test). It was not significantly different among 'non-eaters' and 'eaters' in Mississauga (13.6 vs 8.3 drinks/week; $p=0.17$,Wilcoxon rank sum test) (Table 7-15). Such differences could be important in interpreting liver function results.

Figure 7-6
Smoking distribution
(percentage of participants)

Cornwall (n = 141)



Mississauga (n = 91)

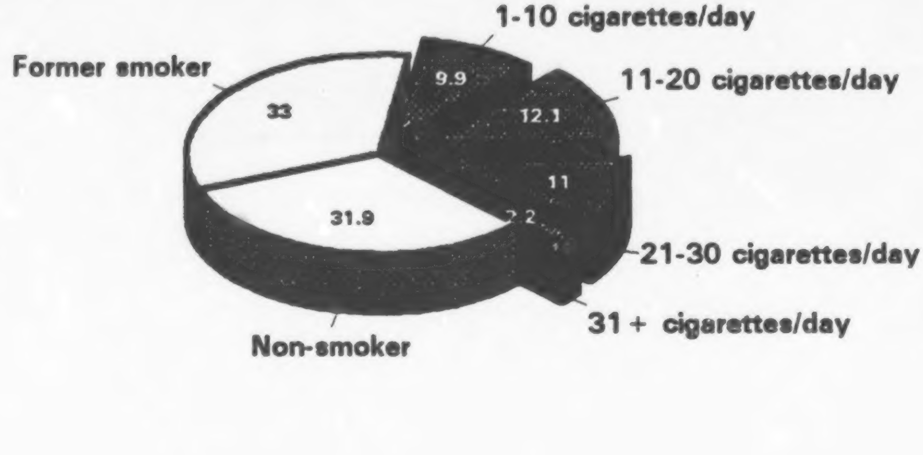


Table 7-15
Potential confounders

(arithmetic means and standard deviations or percentages)

	Cornwall		Mississauga		Both	
	Great Lakes fish		Great Lakes fish		Great Lakes fish	
	"non-eaters" (n = 32)	"eaters" (n = 109)	"non-eaters" (n = 48)	"eaters" (n = 43)	"non-eaters" (n = 80)	"eaters" (n = 152)
Smoking status						
non	37.5%	20.2%	33.3%	30.2%	35.0%	23.0%
former	34.4%	43.1%	33.3%	32.6%	33.8%	40.1%
current	28.1%	36.7%	33.3%	37.2%	31.3%	36.8%
Chew tobacco						
non	96.9%	98.2%	100.0%	97.7%	98.8%	98.0%
former	0.0%	0.9%	0.0%	2.3%	0.0%	1.3%
current	3.1%	0.9%	0.0%	0.0%	1.3%	0.7%
Drinks/week	3.7 (5.2)	6.9 (8.1) (p = 0.023)[1]	13.6 (17.3)	8.3 (9.8)	9.6 (14.6)	7.3 (8.6)
Last drink (days ago)	22.3 (66.9)	8.8 (22.7)	7.3 (27.4)	9.6 (24.0)	12.8 (45.9)	9.0 (23.1)
Taking medication potentially affecting d-glucaric acid levels	0.0%	0.9%	2.1%	7.0%	1.3%	2.6%
Taking thyroid medication	3.1%	3.7%	2.1%	4.7%	2.5%	4.0%

[1] P-value from Wilcoxon rank sum test for differences between "eaters" and "non-eaters"

Few participants took medications known to affect d-glucaric acid (DGA) levels or thyroid medications (Table 7-15). Participants taking medications known to affect DGA levels were excluded for analysis of DGA and fish eating status. However, the use of thyroid medications could be a marker of clinical effect of exposure to environmental contaminants such as PCBs so participants using such medications were included in the analyses of the thyroid data.

A considerable number of self-reported occupational and home chemical exposures were reported (Table 7-16) but few differences were noted between Great Lakes fish "eaters" and "non-eaters". Exposures to resins and isocyanates were significantly more common (p = 0.04) and exposures to coal tar or pitch, significantly less common (p = 0.03), in Mississauga non-eaters compared with eaters. Petroleum products fume exposure was reported more often among Cornwall eaters (p = 0.02). Such exposures may become of concern for sub-clinical effect markers but should not directly affect contaminant levels.

Table 7-16
Percentages of participants reporting occupational and home chemical exposures[1]

	Cornwall		Mississauga		Both	
	Great Lakes fish		Great Lakes fish		Great Lakes fish	
	"non-eaters" (n = 32)	"eaters" (n = 109)	"non-eaters" (n = 48)	"eaters" (n = 43)	"non-eaters" (n = 80)	"eaters" (n = 152)
Question D4: Occupational exposures Metal dust or fumes	25.0	40.4	41.7	53.5	35.0	44.1
Fumes from solvents, paints, glues or varnishes	46.9	44.0	60.4	48.8	55.0	45.4
Resins or isocyanates	18.8	14.7	18.8	4.7 ($p = 0.039$)	18.8	11.8
Pesticides	3.1	11.9	16.7	7.0	11.3	10.5
Coal tar or pitch	3.1	3.7	0.0	9.3 ($p = 0.031$)	1.3	5.3
Fumes from gasoline, diesel, kerosene or fuel oils	25.0	33.0	37.5	34.9	32.5	33.6
Mercury vapours	3.1	1.8	2.1	0.0	2.5	1.3
Electrical transformers	9.4	5.5	8.3	16.3	8.8	8.6
Radiation	3.1	5.5	6.3	9.3	5.0	6.6
Polychlorinated Biphenyls (PCBs)	6.3	4.6	4.2	0.0	5.0	3.3
Ethylene oxide	3.1	0.9	2.1	2.3	2.5	1.3
Explosives	3.1	5.5	2.1	2.3	2.5	4.6
Plastics	9.4	13.8	20.8	14.0	16.3	13.8
Photographic developing chemicals	3.1	1.8	8.3	9.3	6.3	4.0
Question D5: Home exposures Metal dust or fumes	9.4	21.1	25.0	34.9	18.8	25.0
Fumes from solvents, paints, glues or varnishes	75.0	72.5	91.7	86.1	85.0	76.3
Resins or isocyanates	3.1	9.2	16.7	2.3 ($p = 0.022$)	11.3	7.2
Pesticides	40.6	56.9	68.8	60.5	57.5	57.9
Fumes from gasoline, diesel, kerosene or fuel oils	21.9	44.0 ($p = 0.024$)	41.7	34.9	33.8	41.5
Photographic developing chemicals	3.1	1.8	6.3	7.0	5.0	3.3
Question D6: Located within one block of home? Golf course	0.0	0.9	4.2	4.7	2.5	2.0
Dry cleaning plant	3.1	0.9	2.1	9.3	2.5	3.3
Auto repair garage	15.6	10.1	10.4	23.2	12.5	13.8
Parking garage	0.0	0.9	22.9	30.2	13.8	9.2
Highway bridge	3.1	3.7	6.3	11.6	5.0	5.9
Chemical factory	0.0	0.9	0	0	0.0	0.7
Toxic waste or garbage dump	0	0	0	0	0.0	0.0

---

[1] P-values from $\chi2$ statistic testing differences between "eaters" and "non-eaters" have been provided when < 0.05.

Of the potential health conditions which may affect blood lipid levels, 44 people reported having high cholesterol or colitis. In Cornwall only, a higher percentage of Great Lakes fish eaters reported high cholesterol (21.1%) than non-eaters (9.4%), although this was not statistically significant (χ^2 test, $p=0.133$). In Cornwall only, a higher percentage of Great Lakes fish non-eaters (6.3%) reported having colitis than Great Lakes fish eaters (0.0%) (χ^2 test, $p=0.009$). Because these health conditions may affect lipid levels and because lipid levels may affect wet weight levels of organochlorines, all analyses of the relationship between Arochlor 1260 levels and fish consumption and other confounders have been done using the lipid adjusted values.

Thirteen participants reported having a history of hepatitis or liver disease. These conditions could affect the liver plasma enzymes so these people were not included in the analyses of the clinical chemistry results (Section 7.8).

Breastfeeding may be an important route in excretion of lipophilic environmental contaminants such as organochlorines (Jensen 1991). The percentage of all women who had ever breastfed was much higher in Mississauga (66%) than Cornwall (28%) but similar for Great Lakes fish eaters and non-eaters within each community (Table 7-17). The mean number of total months breastfeeding (in lifetime, fully or partially[1]) was similar in both communities and between Great Lakes fish eaters and non-eaters (Table 7-18). It is interesting to look at women who were Great Lakes fisheaters (Cornwall $n=36$, Mississauga $n=17$). The mean number of Great Lakes fish meals and kilograms of Great Lakes fish eaten in the last 12 months were lower in women who had breastfed than women who did not (Table 7-19), although these differences were only significant for number of Great Lakes fish meals in Mississauga. This might indicate that a reduction in consumption during pregnancy (in keeping with Guide recommendations for pregnant women) carried over into non-pregnant and non-breastfeeding years. Of the women who had children, four had changed their consumption of sport fish - two decreased their consumption just prior to their pregnancies, two increased their consumption after their children were born.

---

[1] "Fully" refers to breastfeeding exclusively;
"partial" refers to breastfeeding with other formula or food supplements.

Table 7-17
Number of women who had ever breastfed

	Cornwall			Mississauga			
	Great Lakes fish			Great Lakes fish			
	"eaters"	"non-eaters"	both	"eaters"	"non-eaters"	both	ALL
Had children	32	13	45	11	16	27	72
Breast fed	10	5	15	9	12	21	36
Never breast fed	22	8	30	2	4	6	36
No children	4	5	9	4	1	5	14
All women	36	18	54	15	17	32	86

Table 7-18
Total number of months breastfeeding (lifetime)
(mean (standard deviation)/median/range)

	Cornwall			Mississauga			
	Great Lakes fish			Great Lakes fish			ALL (n=36)
	"eaters" (n=10)	"non-eaters" (n=5)	both (n=15)	"eaters" (n=9)	"non-eaters" (n=12)	both (n=21)	
All months breastfeeding	5.8(4.9) 3.0 1-14	10.5(5.4) 14.0 4.2-15	7.5(5.4) 4.6 1-15	5.5(4.7) 5.0 0.5-15	6.0(5.6) 4.0 1.5-19	5.8(5.1) 4 0.5-19	6.5(5.2) 4.2 0.5-19
Months breastfeeding fully	3.9(2.6) 3.0 1-9	5.7(4.3) 5.0 1.5-13	4.6(3.3) 3.6 1-13	4.9(4.4) 4.5 0.5-15	3.8(2.3) 3.5 0.5-8	4.2(3.3) 4 0.5-15	4.4(3.2) 4 0.5-15
Months breastfeeding partially	1.9(2.8) 0 0-8	4.8(5.9) 2 0-13	2.9(4.2) 1 0-13	0.6(1.1) 0 0-3	2.3(3.8) 0.25 0-12	1.6(3.1) 0 0-12	2.1(3.6) 0 0-13

* one person has missing values

Table 7-19
Breastfeeding status (lifetime) and Great Lakes fish consumption
(arithmetic mean (standard deviation)/median)

	Cornwall		Mississauga		Total	
	Ever breastfed		Ever breastfed		Ever breastfed	
Great Lakes fish meals (in last 12 months)	No (n=26)	Yes (n=10)	No (n=6)	Yes (n=9)	No (n=32)	Yes (n=19)
Number of meals	29.2(40.2) 14.5	24.3(18.3) 20.5	16.7(15.9) 11.0	4.9(3.7) 4.0 ($p=0.05$)	26.8(37.0) 14.5	15.1(16.5) 8.0
Total mass (kg)	5.5(12.5) 1.1	3.8(4.2) 2.4	6.3(8.9) 1.3	1.1(1.0) 0.8	5.6(9.4) 1.2	2.5(3.3) 1.3

7.5 Lipids

Elevated serum lipids were common, particularly among older male participants, in keeping with the endemic nature of this problem in Canada (see ranges in Table 7-20, criteria in appendix 7). The high lipid levels are consistent with participants' reports of having high cholesterol. Also in keeping with this elevation was the body mass index (BMI or weight/height^2) for the anglers, which was distributed as follows: 7 (3%)<20, which may be associated with health problems in some people; 75 (32%) from 20 to 24 inclusive, a good weight for most people; 58 (25%) from 25 to 27 inclusive, which may lead to health problems in some people; and 92 (40%)>27, which puts people at increased risk of developing health problems based on BMI alone (Health and Welfare Canada 1989).

Great lakes fish eaters had slightly higher total lipid levels than non-eaters (Table 7-21) but Great Lakes fish consumption was not related to lipid levels ($p = 0.22$) after accounting for age and gender.

Because we were not carrying out a complete nutritional assessment or modelling potential reductions in heart disease, we did not measure different types of lipids that would be more beneficial and common with increased fish consumption as has been done by others (Dewailly et al 1991). The impact of gender and age on lipids and, hence, serum concentrations of contaminants, is important. Subsequent analyses, therefore, have used the lipid weight basis measures of the organochlorines, in order to detect any independent effect of age or gender on contaminant levels.

Table 7-20
Serum lipids [1]
(Arithmetic mean (standard deviation)/range

	Females (n=86)	Males (n=146) [2]	Both (n=232)
Total Cholesterol (mmol/L)	5.5 (1.0) 2.5-7.5	5.6 (1.0) 3.0-8.0	5.6 (1.0) 2.5-8.0
Free Cholesterol (mmol/L)	1.5 (0.3) 0.5-2.5	1.5 (0.3) 0.7-2.3	1.5 (0.3) 0.5-2.5
Phospholipids (mmol/L)	2.7 (0.4) 1.8-3.5	2.6 (0.3) 1.9-3.3	2.6 (0.3) 1.8-3.5
Triglycerides (mmol/L)	2.1 (1.3) 0.6-9.9	3.1 (2.0) 0.7-13.5	2.7 (1.9) 0.6-13.5
Total Lipids [3] (g/L)	7.8 (1.8) 4.2-16.5	8.7 (2.4) 5.0-19.7	8.4 (2.2) 4.2-19.7

[1] Results are from non-fasted samples.

[2] One person had a missing total cholesterol value and therefore a total lipid value could not be calculated. Another person had a missing free cholesterol and phospholipid value so a total lipid could not be calculated.

[3] Total lipids (g/L) calculated from equation 1 as described in section 6.4.3.2.

Table 7-21
Total serum lipids (g/L)
(arithmetic mean (std. dev.)/range)

Great Lakes fish "non-eaters" (n=79)	8.0 (1.9) (4.2-16.5)
Great Lakes fish "eaters" (n=151)	8.6 (2.3) (5.0-19.7)

7.6 Organochlorine compounds

PCBs

Summary statistics of total PCBs and PCB congener levels in plasma are provided in Table 7-22, by community and Great Lakes fish eating status. Since the data are log-normally distributed geometric means are provided in this table. To facilitate comparison with other studies, arithmetic means from this study and others are provided in Table 7-23. One can see that levels in this study are low in comparison with those observed earlier in Lake

Table 7-22
PCB plasma concentrations (wet weight µg/L)
(geometric mean[1] (geom. standard deviation)/range/ percentage of samples above detection limit)[2]

	Cornwall		Mississauga		All Participants
	Great Lakes fish		Great Lakes fish		
	"non-eaters" (n = 32)	"eaters" (n = 109)	"non-eaters" (n = 48)	"eaters" (n = 43)	
AR1260	3.58 (1.63) 1.30-8.60 100%	4.98 (2.04) 0.74-23.00 100%	3.52 (1.69) 1.10-12.00 100%	4.00 (2.10) 0.93-18.00 100%	4.25 (1.95) 0.74-23.00 100%
TOTAL PCB	1.61 (1.61) 0.59-4.63	2.30 (2.02) 0.43-12.1	1.64 (1.71) 0.55-6.41	1.87 (1.96) 0.46-6.90	1.97 (1.92) 0.43-12.1
PCB28	0.01 (1.89) <0.02-0.07 34%	0.02 (2.04) <0.02-0.14 42%	0.02 (2.18) <0.02-0.17 46%	0.01 (1.89) <0.02-0.11 33%	0.02 (2.03) <0.02-0.17 40%
PCB52	0.02 (1.78) <0.02-0.10 66%	0.02 (1.83) <0.02-0.12 66%	0.02 (1.73) <0.02-0.05 52%	0.02 (1.91) <0.02-0.07 70%	0.02 (1.83) <0.02-0.12 64%
PCB99	0.12 (1.86) 0.03-0.37 100%	0.17 (2.36) 0.02-1.70 100%	0.12 (2.07) 0.03-0.55 100%	0.12 (2.58) <0.02-0.55 98%	0.14 (2.31) <0.02-1.70 100%
PCB101	0.02 (1.70) <0.02-0.06 47%	0.02 (1.95) <0.02-0.17 73%	0.02 (1.83) <0.02-0.07 58%	0.02 (1.91) <0.02-0.08 72%	0.02 (1.92) <0.02-0.17 66%
PCB105	0.02 (2.12) <0.02-0.10 72%	0.04 (2.73) <0.02-0.87 83%	0.03 (2.59) <0.02-0.40 77%	0.03 (2.35) <0.02-0.21 84%	0.03 (2.57) <0.02-0.87 80%
PCB118	0.12 (2.21) 0.03-0.64 100%	0.16 (2.62) 0.02-2.80 100%	0.10 (2.09) 0.02-0.42 100%	0.12 (2.35) 0.02-0.99 100%	0.13 (2.44) 0.02-2.80 100%
PCB128	0.01 (1.00) <0.02-0.01 0%	0.01 (1.41) <0.02-0.08 7%	0.01 (1.22) <0.02-0.04 0%	0.01 (1.00) <0.02 0%	0.01 (1.29) <0.02-0.08 3%
PCB138	0.30 (1.66) 0.10-0.73 100%	0.42 (2.10) 0.06-2.30 100%	0.28 (1.73) 0.08-1.00 100%	0.33 (2.13) 0.08-1.60 100%	0.35 (2.00) 0.06-2.30 100%
PCB153	0.39 (1.61) 0.14-0.92 100%	0.54 (1.99) 0.08-2.30 100%	0.39 (1.67) 0.13-1.40 100%	0.44 (2.10) 0.10-1.80 100%	0.47 (1.92) 0.08-2.30 100%
PCB156	0.06 (1.65) 0.02-0.21 100%	0.09 (2.11) <0.02-0.45 99%	0.06 (1.87) <0.02-0.25 98%	0.07 (2.23) <0.02-0.41 95%	0.08 (2.05) <0.02-0.45 98%
PCB170	0.10 (1.72) 0.03-0.32 100%	0.14 (2.03) 0.02-0.69 100%	0.11 (1.77) 0.03-0.39 100%	0.12 (1.98) 0.03-0.40 100%	0.12 (1.95) 0.02-0.69 100%
PCB180	0.26 (1.78) 0.05-0.89 100%	0.39 (2.00) 0.05-1.90 100%	0.29 (1.80) 0.10-1.90 100%	0.33 (1.95) 0.08-1.30 100%	0.34 (1.95) 0.05-1.90 100%
PCB183	0.03 (1.77) <0.02-0.08 91%	0.05 (2.15) <0.02-0.21 93%	0.03 (2.22) <0.02-0.11 77%	0.04 (2.05) <0.02-0.19 88%	0.04 (2.13) <0.02-0.21 88%
PCB187	0.08 (1.79) 0.03-0.29 100%	0.15 (2.13) 0.02-0.90 100%	0.09 (2.05) <0.02-0.50 96%	0.11 (1.97) 0.03-0.54 100%	0.12 (2.11) <0.02-0.90 99%

1 For the purposes of calculating the geometric mean and standard deviation, non-detectable values have been replaced by a value of 1/2 the corresponding detection limit.

2 Detection limits (µg/L): Ar1260 (0.2), PCB congeners (0.02)

Table 7-23
Comparison of arithmetic mean levels of PCBs from this and other fisheater studies
(μg/L)

	This study[1]		Dewailly et al (1991)[2] Gulf of St. Lawrence Lower North Shore	Fiore et al 1989 & Sonzogni et al (1991)[3] (Wisconsin)	Humphrey (1983)[4] (Lake Michigan)		Hovinga et al (1993)[5] (followup to Humphrey in 1989)		Jacobsen et al (1983)[6] (Lake Michigan women)		Santé Québec (1994) (Québec Inuit)
PCB (total/ congener)	Great Lakes fish non-eaters (n = 80)	Great Lakes fish eaters (n = 152)	fish eaters (n = 195)	anglers (n = 192/173)	controls (1982) (n = 419)	fisheaters (1980) (n = 572)	controls (n = 95)	fisheaters (n = 115)	controls (n = n/a)	fisheaters (n = n/a)	population survey (n = 492)
Aroclor 1260 (all participants)	4.03	6.08	35.2		6.4 (median)	19.6 (median)	6.8	19.0			27.0
Aroclor 1260 (for women of reproductive age only)	2.83 (n = 22)	3.03 (n = 26)							4.1	6.1	
Total PCBs (sum of congeners)	1.86	2.78	12.2	2.2							
PCB118	0.14	0.24	0.87	1.12							
PCB138	0.33	0.52	2.63	1.32							
PCB153	0.44	0.65	3.86	1.46							
PCB170	0.12	0.17	0.80	0.86							
PCB180	0.33	0.47	2.80	1.06							
PCB183	0.04	0.06	0.36								
PCB187	0.11	0.18	0.94	0.98							

[1] Detection limit for Ar1260: 0.2μg/L (all samples greater than detection limit); for PCB congeners: 0.02μg/L (non-detectable values were replaced with a value of 1/2 the detection limit (0.01 μg/L) for calculating the means).

[2] Detection limit for congeners: 0.2μg/L. It is not clear how values below the detection limit were handled when calculating the means.

[3] Detection limit of congeners 0.6μg/L. Congeners found below the detection limit were treated as zero values when calculating the sum of congeners. Means are means of detectable values only.

[4] PCBs were quantified as Aroclor 1260. 99% of values were greater than detection limit of 3μg/L.

[5] PCBs quantified as Aroclor 1260. Detection limit was 3 μg/L. Seven values below d.l. were treated as 2 μg/L.

[6] Of the 196 samples, 44 (22%) were 'detectable but not quantifiable' (below 3μg/L). No details are given as to which participants have missing samples, or how the non quantifiable samples were handled in the analysis. The total number of controls + fisheaters = 196 (no breakdown provided).

Michigan fish eaters in 1982 (Humphrey 1983) and 1989 (Hovinga et al 1993), those observed in Lower North Shore, Gulf of St. Lawrence fishing communities (Dewailly et al 1991) and northern Canadian populations (Santé Québec 1994, Kinloch et al 1992 (means not available for inclusion in Table 7-23)). Mean levels of total PCBs (from sum of congeners) were similar to those reported by Fiore et al (1989) and Sonzogni et al (1991) for Wisconsin anglers. Mean levels for women of reproductive age were lower than those found in Michigan women participants in an earlier study of effects of fish consumption on neurobehavioural development in their children (Jacobson et al 1983, Table 7-23).

Examination of the ranges (Table 7-22) indicates some slightly higher levels, particularly among Great Lakes fish "eaters". Eight Cornwall participants had elevated Aroclor 1260 levels using Health Canada guidance values (see appendix 7). Four participants had Aroclor 1260 levels slightly exceeding the guidance value of 5 μg/L for women of childbearing age (maximum of 7.9 μg/L, two of these people were Great Lakes fish eaters); four participants had levels slightly exceeding the guidance values of 20 μg/L for men and post-menopausal women (maximum of 23 μg/L, all were Great Lakes fish eaters).

Detection of congeners was generally high with most percentages greater than in other studies, presumably due to the low detection limit of 0.02 μg/L in this study. As in other studies, total PCB's from addition of congeners yielded lower levels (40-50%) than Ar1260 measures. These two measures of total PCBs were highly correlated (Table 7-24, Spearman rank correlation=0.98). In fact, Ar1260, total PCBs (by sum of 14 congeners), and congeners 99, 105, 118, 138, 153, 156, 170, 180, 183 and 187 were all correlated with each other with Spearman rank correlations greater than .70 and often higher than 0.85 (keeping in mind that Ar1260 is quantified using peaks 138 and 153). These congeners were also the ones with most participants' values at levels above detection limits. The other congeners were not found above detection limits as frequently (PCB28 - 40%, PCB52 - 64%, PCB101 - 66% and PCB128 - 3%). Correlations are likely to be affected by this truncation of the distribution. Because of the high correlation between Ar1260 and the individual congeners, the 100% detection rate and the use of Ar1260 in the historical literature, Ar1260 was used in the following modelling with the fish and wildlife consumption variables.

Lipid weight basis data are presented in Table 7-25 (as μg/kg lipid). As discussed earlier, the serum lipid adjusted values were used in modelling the (lipophilic) Ar1260 data to take into account differences in serum lipid levels between participants.

Table 7-24
Spearman rank correlations between PCB congeners (in plasma)[1]

	Total[1] PCBs	PCB 28	PCB 52	PCB 99	PCB 101	PCB 105	PCB 118	PCB 128	PCB 138	PCB 153	PCB 156	PCB 170	PCB 180	PCB 183	PCB 187
AR1260	0.98	0.30	0.27	0.89	0.43	0.68	0.79	0.25	0.99[2]	0.99[2]	0.90	0.88	0.91	0.89	0.89
Total[3] PCBs		0.34	0.29	0.89	0.47	0.72	0.82	0.24	0.98	0.98	0.92	0.92	0.93	0.89	0.92
PCB28			0.25	0.30	0.18	0.36	0.38	0.12	0.28	0.29	0.30	0.32	0.29	0.30	0.27
PCB52				0.24	0.42	0.30	0.26	0.18	0.26	0.27	0.28	0.25	0.26	0.28	0.29
PCB99					0.47	0.74	0.80	0.26	0.91	0.88	0.77	0.75	0.72	0.84	0.77
PCB101						0.58	0.52	0.25	0.42	0.44	0.40	0.37	0.39	0.43	0.49
PCB105							0.83	0.25	0.69	0.67	0.62	0.57	0.56	0.66	0.62
PCB118								0.22	0.80	0.78	0.73	0.67	0.68	0.72	0.69
PCB128									0.25	0.24	0.19	0.20	0.20	0.32	0.27
PCB138										0.98	0.89	0.87	0.89	0.89	0.88
PCB153											0.90	0.89	0.93	0.89	0.90
PCB156												0.91	0.91	0.80	0.86
PCB170													0.93	0.80	0.87
PCB180														0.83	0.91
PCB183															0.85

[1] Non-detectable values were replaced with a value equal to 1/2 the detection limit, for purposes of calculating the correlation.

[2] Aroclor 1260 was quantified using the peaks of congeners 138 and 153.

[3] Total PCB's was calculated as the sum of the 14 congeners.

Table 7-25
PCB plasma concentrations based on lipid weight
(µg/kg)

(geometric mean[1] (geom. standard deviation)/range

	Cornwall		Mississauga		All Participants
	Great Lakes fish		Great Lakes fish		
	"non-eaters" (n=32)	"eaters" (n=109)	"non-eaters" (n=48)	"eaters" (n=43)	
AR1260	471.8 (1.53) 213.1-1276.3	590.1 (1.82) 117.1-2430.7	441.6 (1.57) 154.0-1484.3	493.6 (1.92) 142.4-2562.6	521.8 (1.77) 117.1-2562.6
TOTAL PCB	212.4 (1.53) 99.3-643.0	272.4 (1.81) 68.0-1397.1	205.8 (1.59) 75.7-792.9	231.7 (1.8) 70.4-908.3	241.3 (1.74) 68.0-1397.1
PCB28	1.97 (1.82) 0.92-7.38	2.02 (2.06) 0.56-15.8	2.41 (2.05) 0.86-12.0	1.84 (1.85) 0.86-13.4	2.05 (1.99) 0.56-15.8
PCB52	2.46 (1.69) 1.01-12.6	2.48 (1.85) 0.56-12.3	2.17 (1.66) 0.86-6.58	2.91 (1.94) 0.72-7.7	2.48 (1.81) 0.56-12.6
PCB99	15.6 (1.84) 5.05-89.1	20.1 (2.11) 2.62-179.5	14.8 (1.93) 3.85-77.5	14.5 (2.39) 1.50-67.2	17.2 (2.11) 1.5-179.5
PCB101	2.05 (1.83) 0.92-9.8	2.91 (1.88) 0.79-18.0	2.40 (1.82) 0.84-6.80	2.93 (1.81) 1.04-8.40	2.67 (1.86) 0.79-18.0
PCB105	3.27 (2.18) 0.92-24.1	4.77 (2.52) 1.02-100.7	3.92 (2.48) 1.26-55.6	4.27 (2.19) 1.08-25.6	4.26 (2.42) 0.92-100.7
PCB118	15.7 (2.21) 4.58-108.4	18.6 (2.38) 3.40-295.7	12.4 (1.96) 3.66-62.6	14.4 (2.20) 3.04-120.9	16.0 (2.27) 3.04-295.7
PCB128	1.32 (1.27) 0.92-2.41	1.31 (1.46) 0.51-8.45	1.31 (1.31) 0.61-3.51	1.26 (1.27) 0.72-1.98	1.30 (1.37) 0.51-8.45
PCB138	39.8 (1.56) 16.8-115.6	49.4 (1.87) 9.49-242.9	35.7 (1.61) 10.3-118.7	40.6 (1.93) 12.3-227.8	43.3 (1.81) 9.49-242.9
PCB153	51.1 (1.51) 23.6-132.4	64.4 (1.79) 12.7-259.0	49.2 (1.56) 17.97-173.17	54.2 (1.92) 15.3-256.3	57.2 (1.75) 12.7-259.0
PCB156	8.47 (1.56) 3.28-19.8	10.7 (1.89) 1.58-50.7	7.92 (1.75) 1.39-30.92	9.14 (2.03) 1.53-58.4	9.46 (1.86) 1.39-58.4
PCB170	12.9 (1.61) 4.92-30.2	16.5 (1.84) 3.16-77.7	13.1 (1.64) 4.65-45.8	15.1 (1.78) 4.59-51.6	14.9 (1.77) 3.16-77.7
PCB180	34.3 (1.67) 8.20-84.3	46.8 (1.81) 7.91-213.9	35.8 (1.67) 14.1-235.0	41.2 (1.76) 12.3-126.7	41.5 (1.77) 7.91-235.0
PCB183	4.05 (1.67) 1.53-14.5	5.39 (1.94) 1.02-23.7	3.75 (2.01) 1.26-12.6	4.85 (1.88) 1.12-14.4	4.72 (1.93) 1.02-23.7
PCB187	10.9 (1.65) 4.60-27.4	17.2 (1.93) 3.16-101.3	11.5 (1.91) 1.6-61.8	13.8 (1.76) 4.51-46.2	14.3 (1.91) 1.55-101.3

[1] For the purposes of calculating the geometric mean and standard deviation, non-detectable values were replaced with a value of 1/2 the corresponding detection limit.

In order to model the relationship between serum lipid adjusted Ar1260 levels and other variables, a log transformation was used to normalize the data. The primary predictors of interest were

(1) kilograms of Great Lakes fish eaten in last 12 months
(2) number of years eating sport fish
(3) ducks/geese consumption in last 12 months[1]

Age and gender (1 = female, 0 = male) were included in the models as potential confounders. All two-way interactions were included in an initial model with ln(Ar1260) but the only significant ones at the $p = 0.20$ significance level were age x gender and kg-Great Lakes fish x years eaten. These two interaction variables and the main effects variables (listed above) were then included in a stepwise regression model which entered these variables in the following order: age, duck/geese consumption, kg-Great Lakes fish x years eaten, gender and kg-Great Lakes fish. A final model was then constructed (which included main effect variables for all variables involved in interaction terms); the coefficients are provided in Table 7-26.

Table 7-26
Model of ln (Ar1260)
Dependent variable: ln (Ar1260 µg/kg - lipid weight basis)
($r^2 = 0.47$)

Variable	Coefficient (βi)	Standard error (βi)	P-value Type I SS	P-Value Type III SS	$e^{\beta i}$ [2]
Intercept (INTERCEPT)	5.043	0.120			154.9
Age (years) (AGE)	0.025	0.003	0.0001	0.0001	1.025
Gender (GENDER) (female = 1)	-0.145	0.058	0.0001	0.0134	0.865
Duck/geese consumption (in last 12 months) (yes = 1) (DUCKGSE)	0.369	0.079	0.0001	0.0001	1.446
kg-GL fish (in last 12 months) (GLMKG)	-0.011	0.008	0.0055	0.1857	0.989
# years eating sport fish (YRSEATEN)	0.001	0.002	0.1645	0.5446	1.001
kg-GL fish*# years eaten (GLMKG*YRSEATEN)	0.0007	0.0002	0.0087	0.0087	1.0007

---

[1] The variable 'number of duck/geese meals' was highly skewed. An analysis of duck/geese eaters only (n = 39) did not find a relationship between Ar1260 and duck/geese meals (possibly because of the small sample size) consequently a binary (0/1) variable of duck/geese consumption was used in the Ar1260 model.

[2] Since the model is based on the logs of the Ar1260 concentrations, the anti-logs of the variable coefficients (e^{β}) are estimates of the multiplicative effects of the independent variables. For example, the gender coefficient is -0.145; $e^{-0.145}$ is 0.865. Since gender = 1 for females, the model indicates that, on average, females have Ar1260 levels 86.5% of the levels for men (i.e. 14% lower), after adjustment for the other variables in the model.

Clearly age is a very important predictor of lipid-based Ar1260 levels; these levels increased with age. Men had about 16% higher levels than women. Interestingly, duck/geese consumption (yes/no), kilograms of Great Lakes fish (eaten in the last 12 months) and the years eating sport fish are also significant variables in the model. The interaction term "# years eating * 'kg-Great Lakes fish consumed in the last 12 months'" was statistically significant with a positive coefficient. This product term could be seen as an estimate of life-time Great Lakes fish consumption. While these fish and duck/geese consumption variables are significant in the model it must be emphasized that the absolute PCB levels are generally quite low. The model indicates that within these low levels Great Lakes fish and ducks/geese consumption may be contributing to these levels after adjustments for age and gender.

For women, breastfeeding (ever/never) was not significally related to Aroclor 1260 levels after adjustment for the variables in the above model ($p=0.16$). This was based on fitting a model with a variable (0=never breastfed, 1=breastfed at least one child) to the residuals of the model in Table 7-26, for women only.

Similarly, summary measures for cleaning, parts eaten and cooking were not significantly related to Aroclor 1260 levels for Great Lakes fish eaters. This result was based on fitting a model with these variables to the residuals of the model in Table 7-26 for Great Lakes fish eaters only.

For women of reproductive age only, mean lipid based Aroclor 1260 plasma levels were not significantly different for Great Lakes fish eaters (geometric mean 2.72 μg/L, $n=26$) and non-eaters (geometric mean 2.67 μg/L, $n=22$) ($p=0.45$, t-test of logarithms of lipid based Ar1260).

Organochlorine Pesticides

Organochlorine pesticides and their degradation products or metabolites were detected in the serum of a number of participants (Table 7-27 -wet weight/Table 7-28 - lipid weight): DDE, HCB, oxychlordane and transnonachlor were detected in all participants, followed by ß-HCH (99%), mirex (86%), DDT (76%), dieldrin (39%) and heptachlor epoxide (22%). DDE is a metabolite of DDT and reflects historical exposure to DDT. The mean ratio of DDE/DDT was 57.1, ranging from 3.5 to 327.3 (median 39.4) indicating that exposure to DDT was not recent. The chlordanes were rarely detected and then only among sport fish eaters.

Again, arithmetic mean levels were lower than those found earlier in Michigan fisheaters (Humphrey 1983, Hovinga et al 1993), or recently in Gulf of St. Lawrence Lower Shore fishermen (Dewailly et al 1991) and Québec Inuit (Santé Québec 1994) (see Table 7-29). DDE levels were similar to those found by Fiore et al (1989) in Wisconsin anglers. One participant had a level of mirex (1.3 μg/L) towards the upper range of levels extrapolated from a study of Ontario adipose tissue levels (Williams et al 1988) but still considerably lower than the maximum level (4.8 μg/L) seen in the 1992 Québec Inuit survey (Santé Québec 1994). This participant was a Great Lakes fish eater, and one of the 8 participants with slightly elevated PCB levels.

Correlations between levels of organochlorine pesticides (including Ar1260) are provided in Table 7-30. Ar1260 is more highly correlated with DDE (0.72), ß-HCH (0.64), HCB (0.70), mirex (0.60), oxychlordane (0.70) and trans-nonachlor (0.78), than with DDT (0.25), dieldrin (0.32) and heptachlor epoxide (0.32).

Table 7-27
Organochlorine pesticide concentrations in blood plasma (wet weight - µg/L)

**(geometric mean[1] (geom. standard deviation)/range/
percentage of samples above detection limit)**

	Cornwall		Mississauga		All Participants (n = 232)
	Great Lakes fish		Great Lakes fish		
	"non-eaters" (n = 32)	"eaters" (n = 109)	"non-eaters" (n = 48)	"eaters" (n = 43)	
DDE	3.07 (2.82) 0.44-29.0 100%	3.41 (2.59) 0.51-51.0 100%	2.71 (2.29) 0.59-16.0 100%	2.98 (3.36) 0.20-36.0 100%	3.13 (2.69) 0.20-51.00 100%
DDT	0.06 (3.03) <0.03-2.10 84%	0.05 (2.74) <0.03-1.40 71%	0.06 (2.45) <0.03-0.30 77%	0.07 (2.56) <0.03-0.80 84%	0.06 (2.68) <0.03-2.10 76%
β-HCH	0.15 (1.99) <0.03-0.37 97%	0.16 (1.78) 0.05-0.84 100%	0.16 (2.15) <0.03-0.81 98%	0.15 (2.18) 0.03-2.90 100%	0.16 (1.96) <0.03-2.90 99%
HCB	0.18 (1.70) 0.06-0.60 100%	0.22 (1.69) 0.06-1.30 100%	0.20 (1.72) 0.08-1.30 100%	0.21 (1.65) 0.08-0.92 100%	0.21 (1.69) 0.06-1.30 100%
Mirex	0.03 (2.25) <0.02-0.14 75%	0.10 (3.07) <0.02-1.30 93%	0.04 (2.95) <0.02-0.49 79%	0.05 (2.96) <0.02-0.55 84%	0.06 (3.19) <0.02-1.30 86%
Dieldrin	0.07 (1.63) <0.10-0.22 38%	0.08 (1.80) <0.10-0.35 40%	0.08 (1.87) <0.10-0.53 33%	0.09 (1.99) <0.10-0.35 44%	0.08 (1.83) <0.10-0.53 39%
Heptachlor epoxide	0.06 (1.48) <0.10-0.21 19%	0.07 (1.72) <0.10-0.33 26%	0.06 (1.55) <0.10-0.33 19%	0.06 (1.50) <0.10-0.23 19%	0.06 (1.62) <0.10-0.56 22%
Oxychlordane	0.10 (1.98) <0.02-0.28 97%	0.14 (1.91) 0.03-0.73 100%	0.15 (1.68) 0.05-0.70 100%	0.16 (1.86) 0.02-0.52 100%	0.14 (1.88) <0.02-0.73 100%
Trans-nonachlor	0.13 (1.89) 0.03-0.47 100%	0.19 (1.95) 0.03-0.98 100%	0.16 (1.72) 0.06-0.60 100%	0.18 (1.96) 0.04-0.73 100%	0.17 (1.92) <0.03-0.98 100%
α-chlordane	0.01 (1.65) <0.02-0.16 6%	0.01 (1.13) <0.02-0.03 0%	0.01 (1.44) <0.02-0.09 2%	0.01 (1.00) <0.02 0%	0.01 (1.30) <0.02-0.16 1%
γ-chlordane	0.01 (1.64) <0.02-0.12 5%	0.01 (1.13) <0.02-0.03 0%	0.01 (1.22) <0.02-0.04 0%	0.01 (1.00) <0.02 0%	0.01 (1.25) <0.02-0.12 1%

---

[1] For the purposes of calculating the geometric mean and standard deviation, non-detectable values were replaced with a value of 1/2 the coresponding detection limit.

Table 7-28
Organochlorine pesticide concentrations in blood plasma lipids (μg/kg lipid)

(geometric mean[1] (geom. standard deviation)/range

	Cornwall		Mississauga		All participants
	Great Lakes fish		Great Lakes fish		
	"non-eaters" (n=32)	"eaters" (n=109)	"non-eaters" (n=48)	"eaters" (n=43)	
DDE	403.6 (2.70) 72.1-6983.3	404.1 (2.34) 73.8-3315.0	342.6 (2.14) 75.7-1855.3	380.6 (3.14) 22.4-4611.4	386.4 (2.48) 22.4-6983.3
DDT	7.96 (3.06) 1.50-256.5	6.32 (2.54) 1.08-134.8	7.02 (2.25) 1.30-28.1	8.66 (2.22) 1.68-58.9	7.06 (2.50) 1.08-256.5
β-HCH	19.3 (1.78) 2.75-57.8	19.4 (1.57) 7.83-74.5	20.6 (2.05) 1.93-117.6	19.3 (1.99) 4.51-305.7	19.6 (1.77) 1.93-305.7
HCB	23.9 (1.57) 9.84-74.7	25.9 (1.52) 9.74-133.6	25.5 (1.65) 7.62-183.1	27.1 (1.49) 12.3-103.1	25.7 (1.55) 7.62-183.1
Mirex	4.05 (2.18) 0.94-17.1	11.8 (2.84) 0.95-146.4	5.12 (3.02) 0.90-68.1	6.29 (2.67) 1.22-75.6	7.63 (2.99) 0.90-146.4
Dieldrin	9.34 (1.58) 4.72-25.3	9.41 (1.68) 3.92-43.2	9.50 (1.74) 4.22-38.8	11.19 (1.86) 4.55-45.7	9.72 (1.71) 3.92-45.7
Heptachlor epoxide	7.85 (1.51) 4.62-24.1	8.10 (1.61) 3.60-69.1	7.91 (1.44) 4.32-20.0	7.60 (1.43) 3.68-26.9	7.93 (1.53) 3.60-69.1
Oxychlordane	13.5 (1.78) 1.64-28.0	16.7 (1.62) 4.79-47.5	18.4 (1.49) 8.47-46.5	19.9 (1.60) 3.77-48.4	17.1 (1.63) 1.64-48.4
Trans-nonachlor	16.8 (1.65) 5.49-46.7	22.3 (1.70) 4.79-116.2	19.7 (1.54) 9.29-77.8	23.1 (1.72) 7.51-78.6	21.0 (1.68) 4.79-116.2
α-chlordane	1.47 (1.74) 0.92-20.2	1.20 (1.33) 0.51-3.79	1.38 (1.44) 0.61-7.77	1.26 (1.27) 0.72-1.98	1.28 (1.42) 0.51-20.2
γ-chlordane	1.49 (1.71) 0.92-15.2	1.20 (1.33) 0.51-3.79	1.31 (1.31) 0.61-3.51	1.26 (1.27) 0.72-1.98	1.27 (1.39) 0.51-15.2

[1] For the purposes of calculating the geometric mean and standard deviation, non-detectable values were replaced with a value of 1/2 the coresponding detection limit.

Table 7-29
Comparison of arithmetic mean levels of organochlorine pesticides in blood from this and other fisheater studies
(µg/L)

	This study[1]		Dewailly et al (1991)[2] Gulf of St. Lawrence	Fiore et al 1989[3] (Wisconsin)	Humphrey (1983)[4] (Lake Michigan)		Hovinga et al (1993)[5] (followup to Humphrey in 1989)		Santé Québec (1994) (Québec Inuit)
Organochlorine	**Great Lakes fish non-eaters (n=80)**	**Great Lakes fish eaters (n=152)**	**fish eaters (n=25/195)**	**anglers (n=192)**	**controls (1982) (n=419)**	**fisheaters (1980) (n=572)**	**controls (n=95)**	**fisheaters (n=115)**	**population survey (n=492)**
DDE	4.44	5.60	16.4/8.7	6.3 5.3(median)	9.0 (median)	21.7 (median)			11.2
DDT	0.11	0.10			10.5 (median)	26.4 (median)			0.37
DDT + 1.114DDE							6.8.	15.6	
Hexachlorobenzene	0.23	0.25	0.95/0.5		<1	<1			1.42
Dieldrin	0.09	0.10	0.23/0.1		<1	<1			0.23
Mirex	0.06	0.15	0.42/0.2		<2	<2			0.35
Heptachlor epoxide	0.07	0.08	<0.2		<2	<2			0.26
α-chlordane	0.013	0.010	<0.2						0.012
γ-chlordane	0.012	0.010	<0.2						0.033
oxychlordane	0.15	0.18							1.70
transnonachlor	0.17	0.23							2.12
β-HCH	0.20	0.21							0.17

[1] Detection limits (µg/L): DDE,DDT-0.03;HCB,mirex-0.02;dieldrin-0.10. Non-detectable values were replaced with a value of 1/2 the detection limit (0.01 µg/L) for calculating the means.

[2] Detection limits (µg/L): 0.2µg/L. For HCB, dieldrin, DDE and mirex, non-detectable values were replaced with 0.1µg/L. The first number is mean from 25 fishermen, second number (where given) is mean estimated for the study population of 195.

[3] The detection limit for DDE was not calculated but values less than 5µg/L were not reported. 46% of samples had non-detectable levels. It is not clear how non-detectable values were handled when calculating the means.

[4] Detection limits (µg/L): DDE,DDT,HCB,dieldrin: 1; mirex,hept.epoxide: 2. It is not clear if 'Total DDT' is the same as that defined by Hovinga et al, 1992 (i.e. Total DDT = DDE + 1.114DDE).

[5] Detection limit (µg/L) for DDT + 1.114DDE: 1µg/L. All values exceeded detection limit.

Table 7-30
Spearman rank correlations between Aroclor1260, organochlorine pesticides (in plasma), and mercury, lead, cadmium (in whole blood)

	DDE	DDT	β-HCH	HCB	Mirex	Dieldrin	Heptachlor epoxide	Oxychlor-dane	Transnon-chlor	Total mercury in blood	Total mercury in hair - 1^{ST} cm	Lead	Cadmium
AR1260	0.72	0.25	0.64	0.70	0.60	0.32	0.38	0.70	0.78	0.22	0.31	0.41	-0.00
DDE		0.49	0.60	0.65	0.32	0.24	0.34	0.57	0.61	0.10	0.08	0.31	0.03
DDT			0.35	0.35	0.04	0.25	0.34	0.40	0.33	-0.06	-0.07	0.16	-0.02
β-HCH				0.67	0.36	0.29	0.42	0.65	0.65	0.08	0.09	0.27	0.06
HCB					0.40	0.40	0.45	0.76	0.71	0.06	0.15	0.21	0.04
Mirex						0.21	0.17	0.42	0.55	0.24	0.40	0.25	0.03
Dieldrin							0.56	0.45	0.46	0.01	0.08	0.25	-0.01
Heptachlor epoxide								0.45	0.50	0.01	0.11	0.17	-0.01
Oxychlordane									0.85	0.06	0.15	0.34	0.08
Trans-nonachlor										0.17	0.26	0.39	0.01
Total mercury in blood											0.43	0.11	-0.16
Total mercury in hair - 1^{ST} cm												0.11	-0.08
Lead													0.21

Dioxins, dibenzo furans and co-planar PCBs in pooled serum samples

Fourteen pooled serum samples for polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and co-planar PCBs have recently been completed. The methods used to create the pooled samples and calculate the total dioxin equivalency factors (TEQs) are described in section 6.4.3.1.

The results for each pooled sample are provided in Table 7-31 on a lipid weight basis (ng/kg lipid). The total dioxin equivalents (TEQs) are also provided. The co-planar PCB data for Groups 1 (Cornwall) and 9 to 14 (all Mississauga samples) were not obtained due to a faulty batch of adsorbent used by the laboratory.

Preliminary assessment indicates that in Cornwall, levels of dioxins and furans were similar for Great Lakes fish eaters and non-eaters; levels increased with age. Co-planar PCB levels were slightly higher for Great Lakes fish eaters; levels also increased with age. In Mississauga, dioxin and furan levels were slightly higher in Great Lakes fish eaters than non-eaters in the samples for older age categories only. Again, levels increased with age.

Levels of dioxins and furans (and TEQs) found in the pooled samples from the younger age categories were similar to the levels found in a pool of 10 samples collected in 1988 from Red Cross donors from Ontario (Dewailly et al 1994), a group of unexposed firefighters and controls from Quebec (Carrier et al 1990) and levels found in Swedish people eating no fish or moderate amounts of fish (Svensson et al 1991). Dioxin and furan levels (and TEQs) in this study were generally lower than those found in samples from 25 Gulf of St. Lawrence fishermen (Dewailly et al 1994).

Coplanar PCB levels (congeners 126 and 169) for the pooled samples from the younger age categories were similar to levels reported for the Red Cross donor pooled sample from Ontario (Dewailly et al 1994). All samples were considerably lower than levels found in Gulf of St. Lawrence fishermen (Dewailly et al 1994) or Swedish fish consumers (Asplund et al 1993).

Table 7-31
Dioxins, dibenzofurans and co-planar PCB levels in pooled serum samples[1,2]
(lipid based)
(ng/kg lipid)

		Pooled samples													
Community		Cornwall								Mississauga					
Great Lakes fish eating status		'Eaters'					'Non-eaters'			'Eaters'			'Non-eaters		
Age category		<38 years	<38 years	38-50 years	38-50 years	>50 years	<38 years	38-50 years	>50 years	<38 years	38-50 years	>50 years	<38 years	38-50 years	>50 years
Total PCB category		lower	higher	lower	higher	lower	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Pooled sample number		1	2	3	4	5	6	7	8	9	10	11	12	13	14
% lipid		0.73	0.66	0.65	0.80	0.84	0.55	0.68	0.56	0.55	0.75	0.84	0.63	0.70	0.79
Dioxins	TEF[3]														
2378-TCDD	1.0	2.80	2.80	6.42	4.90	5.33	3.04	4.60	5.90	5.40	7.40	4.80	2.80	3.60	ND(3.0)
12378-PnCDD	0.5	6.50	7.00	8.60	10.60	11.80	8.70	6.80	15.10	ND(10.0)	17.00	15.40	ND(9.7)	11.30	11.00
123(4+6)78-HxCDD	0.1	41.60	52.70	68.40	84.50	91.00	55.50	88.10	85.50	56.80	80.10	102.00	44.50	68.10	99.00
123789-HxCDD	0.1	10.00	10.10	13.40	11.50	12.30	14.50	11.30	9.30	12.00	16.00	16.00	11.00	11.00	16.00
1234678-HpCDD	0.01	108.00	62.90	105.00	109.80	131.10	98.40	127.20	126.70	104.00	129.00	80.60	114.00	81.00	115.00
OCDD	0.001	365.30	370.00	566.70	620.60	575.50	417.20	673.10	709.80	422.00	532.20	574.10	530.80	545.70	586.30
Dibenzofurans	TEF[3]														
2378-TCDF	0.1	ND(4.3)[4]	ND(5.4)	ND(4.2)	ND(4.1)	ND(4.3)	ND(4.6)	ND(4.0)	ND(8.0)	ND(7.0)	ND(7.7)	ND(6.0)	ND(7.7)	ND(5.0)	ND(6.0)
23478-PnCDF	0.5	12.30	11.90	11.20	14.80	15.70	13.00	14.90	17.30	24.80	18.50	24.40	15.90	17.40	21.00
123(4+6)78-HxCDF	0.1	10.00	14.6	13.80	15.90	14.20	13.70	16.60	19.80	17.30	20.00	21.00	57.70	13.70	23.00
234678-HxCDF	0.01	5.50	7.90	5.10	4.20	3.80	6.20	4.60	6.50	14.10	9.70	7.80	66.00	ND(5.7)	8.20
1234678-HpCDF	0.01	22.60	18.90	17.00	23.80	18.00	19.20	14.20	19.40	22.40	17.30	17.20	81.30	15.80	18.00
TEQ		20.80	20.78	28.39	31.37	33.49	24.70	29.80	36.78	32.36	40.11	41.23	33.96	29.13	34.34
Coplanar PCBs[5]	TEF[6]														
33'44'-TCB (77)	0.0005	N/A[7]	ND(128.4)	ND(78.2)	ND(67.2)	431.80	ND(244.6)	ND(159.5)	ND(301)	N/A[7]	N/A	N/A	N/A	N/A	N/A
33'44'5-PnCB (126)	0.1	N/A	31.36	25.87	88.60	163.20	21.74	63.71	88.23	N/A	N/A	N/A	N/A	N/A	N/A
33'44'55'-HxCB (169)	0.01	N/A	40.80	46.70	65.90	78.40	36.10	43.60	85.60	N/A	N/A	N/A	N/A	N/A	N/A
TEQ		4.18	3.58	3.07	9.54	17.32	2.60	6.85	9.75	N/A	N/A	N/A	N/A	N/A	N/A
Total TEQ		24.97	24.35	31.46	40.91	50.81	27.30	36.64	46.54	N/A	N/A	N/A	N/A	N/A	N/A

[1]-Acronyms: P-poly; C-chlorinated; DD-dibenzo-p-dioxin; DF-dibenzofuran; B-biphenyl; T-tetra; Pn-penta; Hx-hexa; Hp-hepta; O-octa; TEQ-2378-TCDD toxic equivalents.

[2]-Other PCDFs not detected: 12378-PnCDF, 123789-HxCDF, and 1234789-HpCDF.

[3]-TEFs from Kutz et al 1990; used ½ detection limit for non-detectable values.

[4]-Not detected followed by detection limits in parentheses.

[5]-PCB numbering according to International Union for Pure and Applied Chemistry (IUPAC).

[6]-TEF from Ahlborg et al 1994.

[7]-Values not obtained due to faulty batch of absorbent in laboratory.

7.7 Metals

Mercury

Total mercury levels in whole blood were above detection limit (2µg/L) in 60% of samples and ranged from <2 to 17 µg/L. The arithmetic mean level was 2.6 µg/L; the geometric mean was 2.0 µg/L. Inorganic mercury was detectable in only 19 of the 232 samples (8%), and ranged from <2-3µg/L. Methyl (organic) mercury levels were calculated by subtracting the inorganic mercury level from the total mercury level (when the total mercury level was above the detection limit). The methyl mercury levels in whole blood ranged from 0 to 16 µg/L; on average, methyl mercury accounted for 63% of the total mercury, although this proportion varied widely between individual samples.

Total mercury blood levels are reported by sport fish eating status and community in Table 7-32 (refer back to Table 7-5 for explanation of category). All total mercury blood levels were within the Health Canada 'normal acceptable range' of less than 20 µg/L.

Table 7-32
Total mercury in whole blood
(µg/L)

	Sport fish		
	"Non-eaters"	**"Eaters"**	**Both**
Cornwall			
n	28	113	141
n ≥ detection limit	8	73	81
arithmetic mean (standard deviation)[1]	1.5 (0.8)	2.9 (2.5)	2.6 (2.3)
geometric mean (standard deviation)[1]	1.3 (1.6)	2.2 (2.0)	2.0 (2.0)
range	<2-4	<2-17	<2-17
Mississauga			
n	28	63	91
n ≥ detection limit	16	41	57
arithmetic mean (standard deviation)[1]	2.3 (1.3)	2.8 (1.8)	2.6 (1.6)
geometric mean (standard deviation)[1]	1.9 (1.8)	2.3 (1.9)	2.1 (1.9)
range	<2-6	<2-9	<2-9
Both			
n	56	176	232
n ≥ detection limit	24	114	138
arithmetic mean (standard deviation)[1]	1.9 (1.2)	2.8 (2.3)	2.6 (2.1)
geometric mean (standard deviation)[1]	1.6 (1.8)	2.2 (2.0)	2.0 (2.0)
range	<2-6	<2-17	<2-17

[1] Means and standard deviations were calculated after replacing non-detectable values with a value equal to ½ the detection limit (ie. 1µg/L).

[2] Wilcoxon rank sum test for differences in rank distributions between non-eaters and eaters was significant for males and females separately (both p=0.08) and combined (p=0.0016).

Mean levels were considerably below that found in the Québec Inuit survey (geo. mean = 15.0μg/L, Santé Québec 1994) and in a study of 179 commercial fishermen and their families along the Lower North Shore of the Gulf of St. Lawrence River (geo. mean = 5.4μg/L, Laliberté et al 1992). Levels were also lower than those reported from native populations prior to 1962 (Health & Welfare Canada 1979, 1984). The (arithmetic) mean mercury level for fish eaters in this study (2.8μg/L) is well below the reference value reported by Brune et al (1991) for fish consumers (8.4μg/L) (based on a review of 98 published studies). The mean total mercury level for non-eaters in this study (1.9μg/L) was similar to the reference value for non-eaters (2.0μg/L) (Brune et al 1991).

Statistical modelling was carried out on the natural logarithms of the blood (total) mercury levels. However the results may be unreliable because of the high number of values below the detection limit (which were treated as ½ the detection limit). The following variables were included in a stepwise regression - sport fish consumption, ducks/geese consumption, smoking, age and gender. The resultant model is presented in Table 7-33; the low r^2 of 0.11 may reflect the problem of many non-detectable values. The model indicates blood mercury levels were 35% higher ($p = 0.003$) for sport fish consumers compared with non-consumers after adustment for age and gender. Ducks/geese consumption was not significant ($p = 0.16$) when added to this model.

Table 7-33
Model of ln (total mercury in blood)
Dependent variable: ln (Total mercury in blood - μg/L)
($r^2 = 0.11$)

Variable	Coefficient (βi)	Standard error (βi)	P-value Type I SS	P-Value Type III SS	e^{β}
Intercept (INTERCEPT)	-0.197	0.224			0.82
Age (years) (AGE)	0.017	0.005	0.004	0.0004	1.02
Gender (GENDER) (female = 1)	0.660	0.358	0.015	0.067	1.93
Age * Gender (AGE*GENDER)	-0.019	0.008	0.018	0.016	0.98
Sport fish consumption in last 12 months (1 = yes/0 = no) (SFN12MTH)	0.302	0.099	0.003	0.003	1.35

Hair mercury was analysed in 809 one centimeter samples from the 232 participants. Because of short hair, not all participants were able to provide the 1,2,3,5 and 7 cm segment samples. Total mercury levels were above the detection limit (0.5μg/g) in only 36% of the 809 samples and ranged from <0.5 to 8.4 μg/g (Table 7-34). Inorganic mercury was found above the detection limit (0.5μg/g) in only 17 of the 809 samples (2%) and ranged from <0.5-0.9μg/g. Again, methyl mercury levels were calculated by subtracting the inorganic mercury level from the total mercury level for each sample. Across all the samples, methyl mercury ranged from 0.1 to 7.8 μg/g. Again, on average, methyl mercury accounted for 63% of total mercury.

Mercury concentrations in hair showed good correlation across segments (Table 7-35). The best correlation between blood and hair levels was in the proximal hair segments, in keeping with blood levels better reflecting more recent consumption. Only one participant had a hair mercury level of 8.4μg/g placing him in the "increasing risk" category specified by Health Canada (see appendix 7). (This participant was a fish eater; their blood mercury level was well within the acceptable range).

Geometric mean hair mercury levels were considerably lower (0.28-0.45 μg/g) than levels found in Ontario Amerindians (males 2.7 μg/g, females 2.1 μg/g) (Richardson & Currie 1993) and in Quebec James Bay Cree (9.6 μg/g in 1988, Perrault 1992). Statistical modelling of the hair levels was not carried out due to the large number of non-detectable and missing values. However geometric mean mercury levels were higher in hair samples from Mississauga than in those from Cornwall for sport fish 'non-eaters'. Within the Cornwall samples, mean mercury levels were higher for sport fish eaters than non-eaters. However, in Mississauga mean mercury levels in hair were quite similar for sport fish eaters and non-eaters. These comparisons were not adjusted for any confounding variables.

Mercury levels in blood and hair were not highly correlated with organochlorine blood levels (refer back to Table 7-30). Indeed, mercury tends to be higher in fish from some inland lakes while organochlorines are higher in some Great Lakes locations.

Table 7-34
Total mercury in hair
(μg/g)

	Sport fish 'non-eaters' (n = 56 participants)					Sport fish 'eaters' (n = 176 participants)				
	Centimeter section					Centimeter section				
	1	2	3	5	7	1	2	3	5	7
Cornwall										
# ≥ 0.5μg/g	3	3	4	1	1	54	52	41	16	8
# < 0.5μg/g	25	25	22	16	10	59	59	49	26	14
number missing (no sample)	0	0	2	11	17	0	2	23	71	91
arith. mean	0.31(0.20)	0.30(0.14)	0.32(0.19)	0.28(0.11)	0.29(0.14)	0.65(0.92)	0.63(0.92)	0.59(0.79)	0.41(0.24)	0.40(0.22)
geo. mean	0.28(1.44)	0.28(1.38)	0.29(1.48)	0.27(1.28)	0.27(1.36)	0.45(2.10)	0.44(2.07)	0.43(2.01)	0.36(1.63)	0.36(1.64)
median	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
range	<0.5-1.2	<0.5-0.8	<0.5-0.9	<0.5-0.7	<0.5-0.7	<0.5-8	<0.5-8.4	<0.5-6.6	<0.5-1.3	<0.5-0.8
Mississauga										
# ≥ 0.5μg/g	6	9	4	4	5	22	23	19	9	5
# < 0.5μg/g	21	18	20	9	3	41	40	32	20	11
number missing (no sample)	1	1	4	15	20	0	0	12	34	47
arith. mean	0.45(0.44)	0.51(0.44)	0.38(0.31)	0.38(0.22)	0.48(0.23)	0.40(0.25)	0.43(0.30)	0.46(0.34)	0.41(0.27)	0.38(0.22)
geo. mean	0.35(1.90)	0.39(1.99)	0.31(1.69)	0.34(1.62)	0.43(1.64)	0.35(1.63)	0.37(1.72)	0.38(1.79)	0.35(1.72)	0.34(1.60)
median	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
range	<0.5-1.9	<0.5-1.6	<0.5-1.5	<0.5-0.9	<0.5-0.9	<0.5-1.5	<0.5-1.5	<0.5-1.7	<0.5-1.0	<0.5-1.0

[1] Means and standard deviations were calculated after replacing all non-detectable values with a value equal to ½ the detection limit (ie. 0.25μg/g).

Table 7-35
Spearman correlations between total mercury measurements in blood and hair[1]

		Total mercury in hair				
		cm 1	cm 2	cm 3	cm 5	cm 7
Total mercury in blood		0.43	0.41	0.37	0.33	0.11
Total mercury in hair	cm 1		0.83	0.68	0.64	0.62
	cm 2			0.78	0.76	0.62
	cm 3				0.80	0.56
	cm 5					0.71

[1] Correlations were calculated after replacing non-detectable values with a value equal to 1/2 the detection limit.

Lead

A total of 201 blood samples were analysed for lead (Table 7-36). Thirty-one samples (13%) were clotted[3]. Blood lead levels were generally low, ranging from <0.5μg/dL to 26.8μg/dL. Two samples (1%) were non-detectable. Only one man had a blood lead level slightly above the Federal-Provincial Committee on Lead's guideline of 25 μg/dL. He had worked as a mechanic for many years. All women of reproductive age had levels below 10 μg/dL, the maximum level recently recommended by the Working Group on Blood Lead Intervention Levels and Strategies (1995, in press), for protection of the foetus.

Table 7-36
Lead in whole blood (μg/dL)
(geometric mean[1] (standard deviation)/range/n)

	Sport fish	
	"non-eaters"	"eaters"
Cornwall Females	1.9 (2.3) <0.5-6.2 10	2.4 (1.8) <0.5-5.6 32
Males	3.2 (1.4) 1.9-4.7 9	4.2 (1.6) 1.1-10.3 61
Mississauga Females	2.7 (1.5) 1.4-4.4 11	3.0 (1.7) 1.1-7.4 21
Males	3.6 (1.5) 1.8-7.3 16	4.5 (1.7) 1.8-26.8 41

[1] Non-detectable values were replaced by values = ½ detection limit (ie. 0.25 μg/dL) for calculation of means and standard deviations.

Arithmetic mean levels were slightly lower in this study (3.5 μg/dL for sport fish non-eaters; 4.3 μg/dL for eaters) than those found by Hovinga et al (1993) in a study of Michigan fisheaters and controls (3.8 μg/dL for non-eaters, 5.5 μg/dL for eaters) and considerably lower than the mean levels found in 1) a population survey of Québec Inuit in 1992 (10.1 μg/L) (Santé Québec 1994), 2) Red Cross blood donors in Alberta in 1980 (10.0 μg/dL), 3) radiator shop workers in Alberta in 1983 & 1986 (33.3 μg/dL and 42.71 μg/dL respectively (Mah 1990)), 4) Swiss men (13.1 μg/dL) and women (9.1 μg/dL) (Berode et al 1991) and 5) a Belgian population survey in 1988 (8 μg/dL) (Ducoffre et al 1990).

---

[3] The clotted samples could not be analysed for lead and cadmium. They occurred more often in Cornwall then Mississauga. This suggested technician error (insufficient mixing of blood in sample tube), but the clotted samples occurred across all technicians in Cornwall. Another possibility is a poor batch of tubes with insufficient anti-coagulant.

The distribution of lead values was non-normal. No transformation could be found to normalize the data completely. However a (natural) logarithmic transformation produced a more symmetrical distribution than the raw data, hence the logs were used for further analysis. Geometric means are provided in Table 7-36. The (geometric) mean levels were higher among Great Lakes fish eaters than non-eaters and among men than women. Blood lead levels were also somewhat correlated with organochlorine levels (Table 7-30; Spearman rank correlation with Ar1260 was 0.41), suggesting that a significant portion of the lead may have been derived from a common source.

The results of a regression analysis are presented in Table 7-37. An age x sport fish consumption interaction term was significant at p=0.03 but was not included in the model presented in Table 7-37 to simplify interpretation (parameter estimates were similar). Sport fish consumption (0/1) and kilograms of sport fish eaten were found to be significantly associated with ln(lead), after adjustment for age, gender, community and smoking.

Table 7-37
Model of ln (lead in whole blood)
Dependent variable: ln (lead $\mu g/L$ - in blood)
($r^2=0.34$)

Variable	Coefficient (βi)	Standard error (βi)	P-value Type I SS	P-Value Type III SS	e^{β}
Intercept (INTERCEPT)	0.477	0.155			1.61
Age (years) (AGEINT)	0.015	0.003	0.0001	0.0001	1.02
Gender (female=1) (GENDER)	-0.495	0.071	0.0001	0.0001	0.61
Community (Mississauga=1) (COMMUN01)	0.164	0.068	0.0221	0.0167	1.18
Current smoking (yes=1) (SMOKENOW)	0.246	0.072	0.0003	0.0008	1.28
Sport fish consumption (in last 12 months) (1=yes) (SF12MTH)	0.140	0.083	0.0271	0.0943	1.15
kg of sport fish eaten (in last 12 months) (SFMKG)	0.005	0.003	0.0748	0.0748	1.005

Hovinga et al (1993) has also found lead levels higher in Great Lakes fish eaters compared with non eaters. Similarly, in the survey of Québec Inuit, lead levels increased with consumption of marine mammals (however a multifactorial analysis was not presented) (Santé Québec 1994). Consumption of sport fish is not expected to be a major route of exposure to lead since lead levels are generally very low in Ontario sport fish (Cox C, pers.

commun.). In fact, the blood levels found in this study are generally low. Other sources of lead may be associated with sport fishing, including use of lead sinkers, home canning of fish (with lead soldered cans) or with related consumption of game and wildfowl with accidental ingestion of lead shot. Indeed, it is difficult to separate the effects of sport fish consumption ducks/geese consumption and game/wildfowl consumption variables. Game consumption in the last 12 months (yes/no) and ducks/geese consumption in the last 12 months (yes/no) are not significant when added to the model in Table 7-36. However, both of these variables are significant ($p = 0.03$ for game/wildfowl; $p = 0.08$ for ducks/geese) when they replace the sport fish consumption variables. The results do point to the need to consider lead levels when carrying out neurobehavioural studies of fish and wildlife eaters or their children.

Cadmium and urinary cotinine

The results from the analyses of cadmium in whole blood are presented in Table 7-38, 7-39, and Figure 7-7. The cadmium data were affected by a large number of clotted samples (13%, see earlier comments regarding the lead analyses). As well, a significant proportion of samples had levels below the detection limit (37% of useable samples) (Table 7-38). Levels ranged from <0.2μg/L to 14.6μg/L.

Table 7-38
Cadmium in whole blood (μg/L) - sample status and percentages
(number of samples)

	Females	Males	Both
Unuseable (clotted)	12 (14%)	19 (13%)	31 (13%)
Useable, ≥ detection limit (0.2 μg/L)	56 (65%)	71 (49%)	127 (55%)
Useable, < detection limit (0.2 μg/L)	18 (21%)	56 (38%)	74 (32%)
TOTAL	86 (100%)	146 (100%)	232 (100%)

Cadmium in blood generally reflects current exposure (within the last few months) (Lauwerys & Hoet 1993). In this study, the overwhelming factor affecting blood cadmium levels was smoking status (see Table 7-39, Figure 7-7 and regression model in Table 7-40). Mean levels steadily increased with increasing numbers of cigarettes, as observed in other studies (Dewailly et al 1991, Hovinga 1993, Nieboer et al 1988). Sport fish consumption and deer/moose consumption were not significantly related to blood cadmium levels.

Table 7-39
Cadmium in whole blood ($\mu g/L$) by smoking status

(geometric mean (standard deviation), range, n)

	Females (n = 74)	Males (n = 127)	Both (n = 201)
Non-smokers	0.17 (2.03) <0.2-0.9 22	0.12 (1.59) <0.2-0.8 37	0.14 (1.79) <0.2-0.9 59
Ex-smokers	0.37 (2.80) <0.2-3.4 22	0.25 (2.75) <0.2-3.0 55	0.28 (2.79) <0.2-3.4 77
Current smokers **1-10 cigarettes/day**	3.64 (1.46) 2.1-5.0 4	2.43 (1.96) 1.0-6.1 10	2.72 (1.86) 1.0-6.1 14
11-20 cigarettes/day	4.43 (1.53) 1.7-8.7 13	3.31 (1.89) 1.2-7.2 8	3.97 (1.69) 1.2-8.7 21
21-30 cigarettes/day	4.24 (1.59) 1.7-6.7 10	4.07 (1.46) 2.4-8.6 14	4.14 (1.50) 1.7-8.6 24
≥ 31 cigarettes/day	8.35 (2.10) 3.6-14.6 3	6.80 (1.26) 5.9-8.9 3	7.54 (1.66) 3.6-14.6 6
All current smokers	4.53 (1.64) 1.7-14.6 30	3.50 (1.81) 1.0-8.9 35	3.94 (1.75) 1.0-14.6 65

Figure 7-7

Cadmium in whole blood (μg/L) by smoking status

(geometric mean with minimum and maximum values)

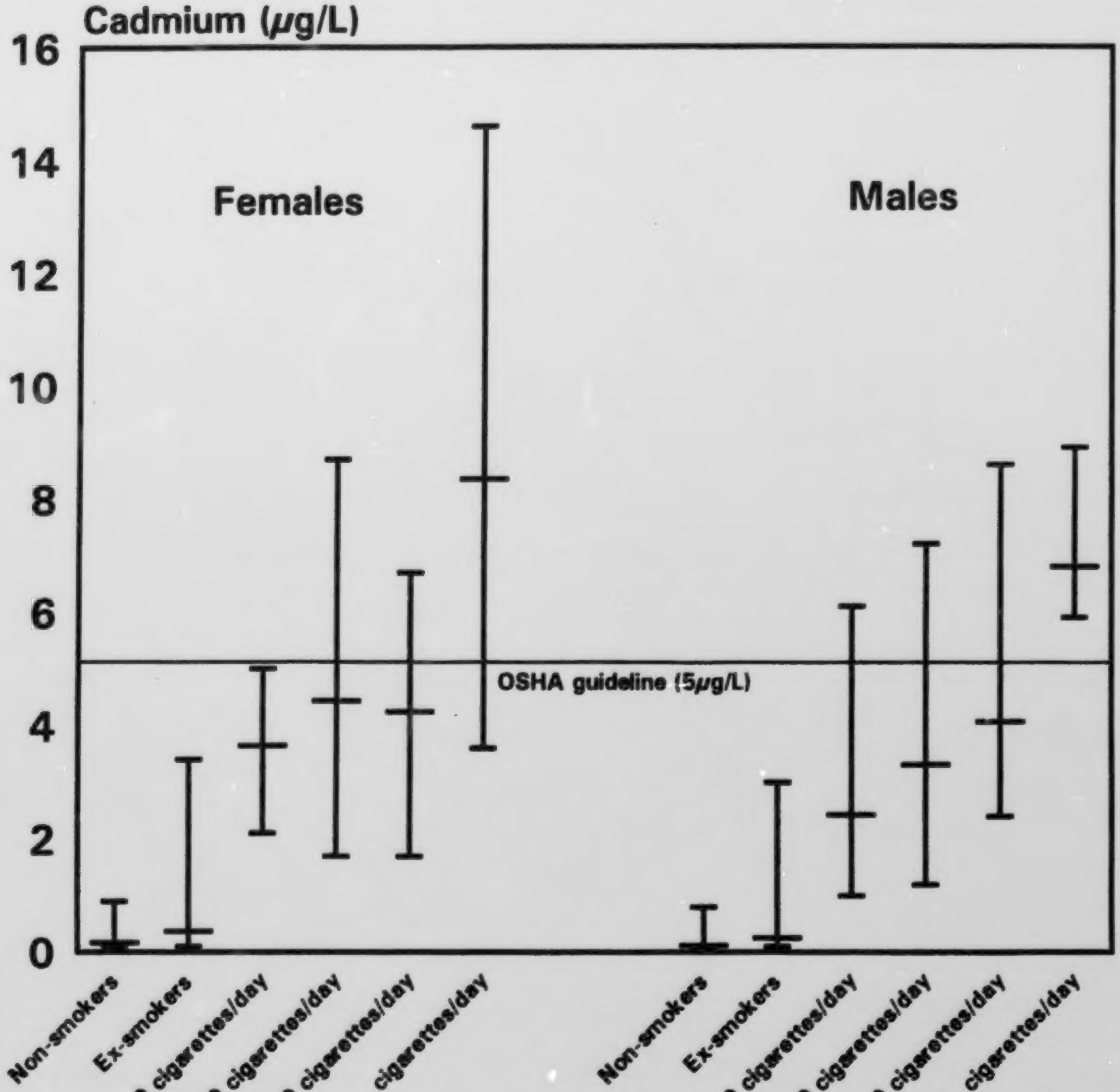


Table 7-40
Model of ln (cadmium in whole blood)
Dependent variable: ln (cadmium) in blood - μg/L)
($r^2 = 0.76$)

Variable	Coefficient (βi)	Standard error (βi)	P-value Type I SS	P-Value Type III SS	$e^{\hat{\beta}}$
Intercept (INTERCEPT)	-2.202	0.235			0.111
Current smoker (1 = yes) (SMOKENOW)	2.453	0.231	0.0001	0.0001	11.62
Number of cigarettes/pipes smoked/day (NUMSMOKE)	0.025	0.010	0.0056	0.0131	1.025
Age (years) (AGE)	0.012	0.005	0.0146	0.0169	1.012
Gender (1 = female) (GENDER)	0.253	0.118	0.0334	0.0334	1.288

The geometric mean of the cadmium levels in blood for all current smokers was 28 times that of non-smokers (3.94μg/L vs 0.14 μg/L). The levels are similar to those found in other Canadian studies (Gulf of St. Lawrence fishermen (Dewailly et al 1991) and Québec Inuit (Santé Québec 1994)) but the levels in smokers in this cohort are considerably higher than those levels found by Hovinga et al (1993) in a study of Wisconsin Great Lakes anglers. In that study, the arithmetic means for smokers smoking 1-30 pack years were 0.52 μg/L (fisheaters) and 0.41 μg/L (controls). For smokers smoking more than 30 pack years they were 1.03 μg/L (fisheaters) and 0.65 μg/L (controls). The maximum cadmium level was 5.0 μg/L. Levels found in this study are clearly higher than these levels (geometric mean of 7.54 μg/L for smokers smoking more than 31 cigarettes/day, maximum value of 14.6). These results may reflect higher cadmium levels in Canadian cigarettes, such as was found by Watanabe et al (1987). In that study Canadian cigarettes had a mean cadmium level of 2.01 μg/g cigarette compared with 1.48 μg/g for American cigarettes.

Twenty-five participants (all smokers) had cadmium levels above the U.S. Occupational Safety and Health Administration guideline of 5 μg/L (see appendix 7). The person with the highest level of 14.6 was one of the heaviest smokers but also consumed 50 moose meals per year, including 3-4 moose livers. Moose are known to accumulate cadmium in liver and kidney. Based on the regression model in Table 7-40, it is estimated that smoking, age, and gender accounted for about 3/4 of this value. Blood cadmium levels were not correlated with organochlorine levels (Table 7-30), presumably because in this cohort the primary source of cadmium is smoking.

Urinary cotinine

Statistics on the urinary cotinine levels for smokers, former smokers and non-smokers are presented in Table 7-41 and Figure 7-8. A strong relationship between urinary cotinine and smoking was found; levels in smokers were over 200 times that of non-smokers. However, geometric mean levels of urinary cotinine did not increase consistently with increasing number of cigarettes per day.

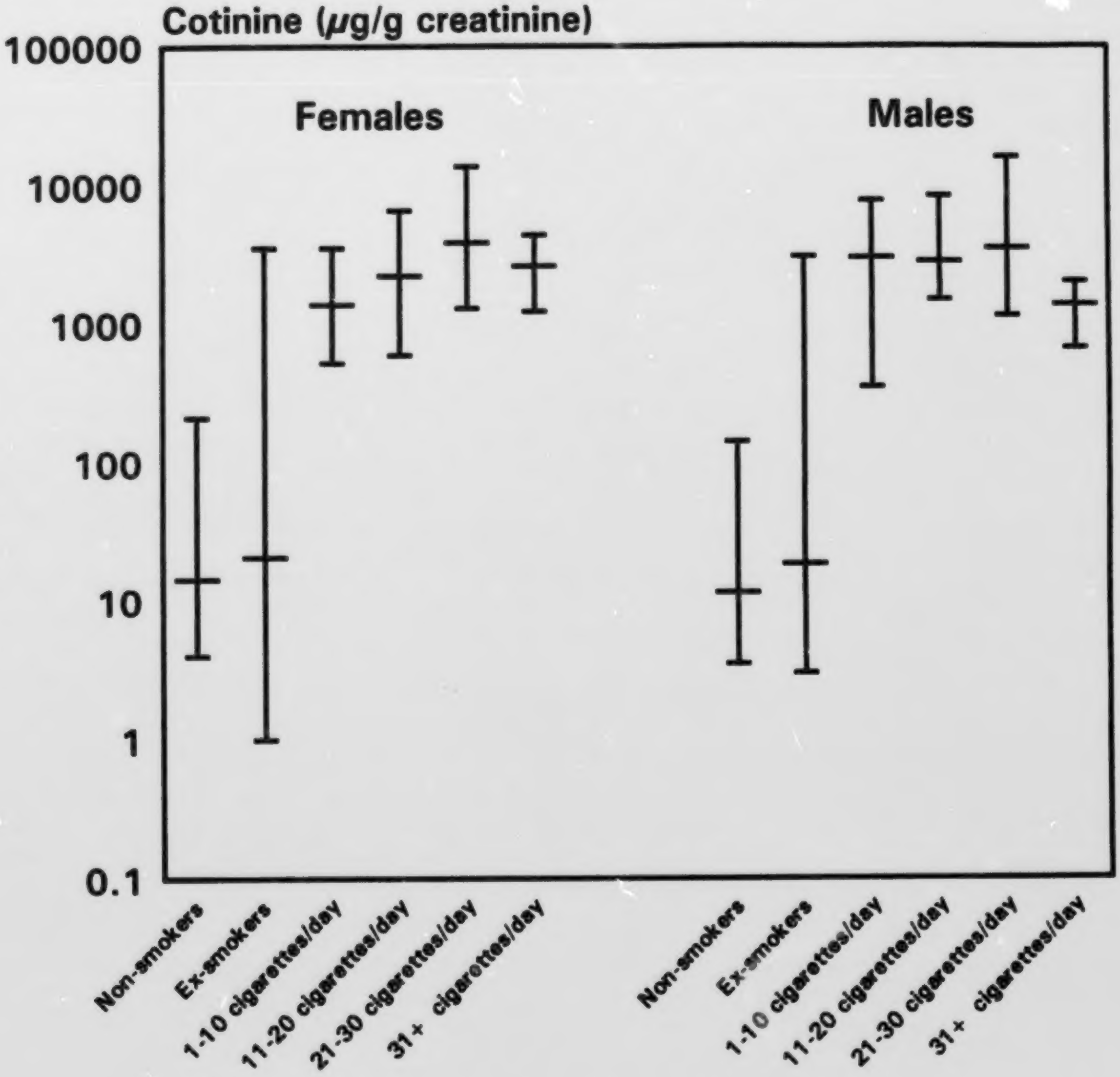
Table 7-41
Urinary cotinine
(µg/g creatinine)
(geometric mean (standard deviation)/range/n)

	Females (n = 74)	**Males (n = 127)**	**Both (n = 201)**
Non-smokers	14.3(3.7) 4-210 23	11.5(3.6) 3.5-140 40	12.4(3.7) 3.5-210 63
Ex-smokers	20.7(9.4) 1-3510 27	18.4(5.7) 3-3045 61	19.0(6.7) 1-3510 88
Current smokers			
1-10 cigarettes/day	1370.5(2.2) 520-3510 4	2963.3(2.3) 345-7665 11	1559.8(2.2) 345-7665 15
11-20 cigarettes/day	2208.3(1.9) 595-6555 13	2776.9(1.6) 1470-8240 12	2465.0(1.7) 595-8240 25
21-30 cigarettes/day	3840.0(2.1) 1290-13580 16	3463.3(2.0) 1130-15740 17	3641.0(2.0) 1130-15740 33
> = 31 cigarettes/day	2596.5(2.0) 1220-4335 3	1352.5(1.6) 660-1990 4	1788.7(1.9) 660-4335 7
All current smokers	2714.5(2.1) 520-13580 36	2481.5(2.1) 345-15740 44	2583.7(2.1) 345-15740 80

Figure 7-8

Urinary Cotinine (μg/g creatinine) by smoking status

(geometric mean with minimum and maximum values)



Smoking status

7.8 Clinical chemistry - thyroid status and liver enzymes

Clinical chemistry analyses produced a large number of abnormal results that had to be interpreted individually and reported to the participants and their physicians (see appendix 7). Six and a half percent of participants had abnormal thyroid hormone plasma levels or were on thyroid medication but this was not associated with Great Lakes fish eating status in either community.

Seventeen percent of participants had one or more liver plasma enzyme levels outside the normal range. After removing the 13 participants who reported liver disease or hepatitis, this percentage was not statistically different for Great Lakes fish eaters vs non-eaters in either community. Abnormal liver enzyme plasma levels were strongly related to the number of alcohol drinks per week ($p=.0001$). The average number of drinks per week for participants with one or more enzyme level outside of normal range was 15.12 ($n=51$, standard deviation = 17.19), compared with 6.13 for participants who did not have any abnormal enzyme levels ($n=181$, standard deviation = 7.6).

7.9 Sub-clinical effect markers - D-glucaric acid and urinary porphyrins

Eleven percent of participants had one or more urinary porphyrin level outside of normal ranges; no relationship with fish consumption was found.

D-glucaric acid levels ranged from 1.41 to 655.53 μmoles/g creatinine, with a mean level of 49.2 μmoles/g creatinine. No relationship was found with fish consumption or contaminant levels.

7.10 Physician feedback on individual results dissemination

Some participants (7) did not have physicians and two sets of patients shared a physician (2x2). This resulted in 13 physicians being contacted in each of the two communities (total of 26) for the 35 participants with contaminant levels above our guidelines.

Repeated contacts with participants and physicians were often required due to the following reasons: the participant had changed physicians (3), physician had limited office hours (8), patient had not been seen yet (3), chart was not at designated office (2), physician was busy and would call back (5) and physician had not received letters (5). Interviews could not take place when no correct phone number could be obtained (2), the physician could not identify the participant(s) as their patient(s) (3) or patient follow up had not occurred prior to the end of the survey (3). Final physician contact rate was 69% overall, slightly higher in Mississauga (10/13) than in Cornwall (8/13).

Responses from physicians

Summaries of the comments provided by the physicians are given below:

1. Q: Was the information provided to the physician clear?

   A: Most physicians found the presentation of contaminant information clear. One said he learned a lot reading it. Another very much liked the presentation in table format. None found it too detailed or superficial but two had not read it. One was uncertain whether there was any cause for real concern and another wondered what to do about the elevated contaminant levels.

2. Q: Did physicians discuss the information with the participant/patient?

   A: Eleven physicians (42%) had discussed the contaminant information with their patients. Most of the rest had been unable to see the patient yet, but two had not seen any particular reason to see their patients about it. Many focused more easily on the clinical laboratory results which had come out earlier.

   Most patients were glad that their levels were not high enough to cause much concern. One woman with PCB levels above guidelines for childbearing women was close to menopause and not planning on further childbearing. Several patients were willing to limit their fish consumption as advised. Two physicians spoke of the usefulness of the Health Canada handout, "Having your catch and eating it too!", in counselling their patients. Smoking reduction was another step that some participants and their physicians discussed to reduce cadmium levels but several were unaware of the connection between cadmium and smoking despite our information.

3. Q: What follow up of test results occurred?

   A: Six physicians ordered repeat clinical tests. Most of these repeat tests resulted in lower levels, but three patients still required treatment changes: lower fat diet, reduction in alcohol consumption and adjustment of thyroid medication. Two physicians ordered repeated tests for contaminant levels: one for PCB's, and one for urinary cadmium (plus kidney function tests).

4. Q: Any further information or activities to health professionals that physicians could suggest?

   A: Most physicians were satisfied with the information provided to date and saw no further need. One had training in occupational health and spoke comfortably about the contaminant data. Several asked questions of the medical advisor during the telephone interview, seeking guidance on severity of the contaminant problem and appropriate management. Three wanted ongoing information (2 in Cornwall, 1 in Mississauga). One Cornwall physician wanted posters to put up in his office and the local hospital and to share with colleagues. This physician and an occupational health nurse were interested in attending a meeting presenting the group results.

8.0 CONCLUSIONS AND RECOMMENDATIONS

1. Participation rates were lower than expected. Potential participants who declined to participate were concerned with blood sampling and lack of personal benefit from the study. In future field studies, greater emphasis should be placed on dealing with these fears, concerns and lack of personal interest or the studies should be conducted through clinical settings which typically experience higher response rates.

2. Participation rates were higher for men than women and higher for sport fish eaters than non-eaters (controls). Participation rates were also higher in the higher income group. Future studies should investigate methods to encourage greater participation in the under-represented groups.

3. Consumption of sport fish was comparable to some other studies in the Great Lakes basin, however consumption of Great Lakes/St. Lawrence fish particularly in Mississauga was not especially high. Fish consumption in both Cornwall and Mississauga was much lower than that seen in the subsistence fishing population on the North Shore of the St. Lawrence River by Dewailly et al 1991. Either the fish license holder sample frame was not an effective method of reaching high fish consumers, or there are few people consuming Great Lakes fish at high levels.

4. There were significant differences between participants in Cornwall and Mississauga with respect to species of fish, fishing locations, and frequency and size of sport fish meals eaten. Generally, meal sizes were larger in Mississauga than Cornwall, but Cornwall participants ate Great Lakes fish more often. Cornwall participants primarily ate perch, walleye and bass from the St. Lawrence River and Eastern Lake Ontario. Mississauga participants ate predominantly bass, rainbow trout and walleye from inland lakes and salmon and rainbow trout from the Great Lakes. In future studies, such community differences must be considered in the study design (eg. fish consumption questions) and in the data analyses.

5. Many participants had been eating sport fish for decades. This has important implications for body burdens of persistent contaminant levels, and needs to be considered in future sport fish consumption studies.

6. Awareness of the 'Guide to Eating Ontario Sport Fish' was higher in Mississauga than

Cornwall, and in both communities, was related to education and income levels. Overall, 42% of participants were unaware of the Guide or never followed the advice in the guide. More research into the effectiveness of the guide and other methods of informing potential high fish consumers about the consumption of sport fish is recommended.

7. Mean PCB (as Aroclor 1260) blood plasma levels were well below mean levels seen in other fish eating studies. For most participants, levels were below Health Canada PCB blood guidance values. Four participants had Aroclor 1260 levels slightly exceeding Health Canada's guidance value of 5μg/L for women of childbearing age. Four participants had levels slightly exceeding the guidance value of 20μg/L for men and post-menopausal women. For some of these participants, Great Lakes fish consumption may have contributed to these elevated levels.

8. Within the relatively low levels of PCBs found in the study, there was a significant relationship between lipid adjusted Aroclor 1260 levels and duck/geese consumption (yes/no) and Great Lakes fish consumption (kg of Great Lakes fish eaten in the last 12 months and # years eating sport fish) after adjustment for age and gender.

9. Many sport fish eaters also ate game and wildfowl. Given the relationship found with PCBs levels and ducks/geese consumption, the assessment of waterfowl consumption should be included in future studies of fish eaters.

10. Since organochlorines are lipophilic, blood lipid levels may affect organochlorine levels. Wet weight data are useful for interpretation of data on an individual level (i.e. toxicity assessment). Lipid adjusted values are necessary for comparing levels across groups, especially since this study found that fisheaters had higher blood lipid levels.

11. Aroclor1260 (quantified from congeners 138 and 153) and total PCB levels in blood serum (from the sum of 14 congeners) were highly correlated ($p = 0.98$). These variables were also highly correlated with those congeners which had few non-detectable values (generally, the higher chlorinated ones). Statistical analysis of the other congeners was difficult due to the high number of non-detectable values. Consequently, it is concluded that Ar1260 measurements were sufficient for assessment of overall PCB exposure in this population. This comment does not include co-planar PCBs.

12. Mean blood plasma levels of organochlorine pesticides were lower than mean levels seen in other fisheating studies. The mirex level of one participant appeared to be slightly higher than expected based on extrapolation of levels found in autopsy fat studies in Ontario (this person also had an elevated PCB level), but was much lower than the maximum value seen in a recent survey of Québec Inuit. Interpretation of organochlorine plasma levels is difficult because of the limited number of blood guidelines. The good correlations between detectable organochlorine pesticides and Arochlor 1260 suggest that PCB levels are also a reliable indicator of organochlorine pesticide levels.

13. Comparisons of PCB levels (and other contaminants) between studies are complicated by a number of factors:
    - Technological improvements have resulted in lower detection limits now than in earlier studies (for example, 0.02 μg/L in this study, 0.2μgL in Dewailly et al (1991), 0.6μg/L in Sonzogni (1993) and 3μg/L in Hovinga (1993) and Humphrey (1983). As well, some studies differ in the way non-detectable values are handled when calculating means and other statistics.
    - In recent studies, total PCBs are calculated as sums of individual congeners, but studies differ in the kinds and numbers of congeners analysed and used for calculating the sum. As well, how non-detectable values are handled in the sum can affect the total PCB value, making comparisons difficult.

    If the number of non-detectable congeners is high, the statistical handling of these values should be clearly stated. It is useful to have data reported as Aroclor 1260 to facilitate comparison with earlier studies. This recommendation does not refer to coplanar PCBs.

14. Levels of dioxins, dibenzofurans and coplanar PCBs (and total dioxin equivalents (TEQs)) in 14 pooled samples were also low, similar to other studies of people with low to moderate fish consumption. Levels appear to be most strongly related with age. levels of dioxins and dibenzofurans were similar for Great Lakes fish eaters and non-eaters except in Mississauga among older participants. Coplanar PCB levels were slightly higher for Great Lakes fish eaters then non-eaters.

15. Total blood mercury levels in this study population were very low (40% of samples were below detection limit) and levels were below Health Canada's guidance value. On average, methyl mercury accounted for 63% of total mercury. Total blood mercury levels were related to sport fish consumption (yes/no) after adjustment for age and gender.

16. Levels of mercury in hair were generally very low, with only 36% of samples above the detection limit. With the exception of one person, all values were below Health Canada's guidance value. Given the cost of analysis, this does not appear to be a useful measure for studies of low exposure populations.

17. Blood lead levels were low (except for one participant) and below current blood guidelines. Lead levels were 29% higher in Mississauga than Cornwall. A relationship was also found with sport fish consumption (yes/no and kilograms of sport fish eaten in last 12 months) after adjustment for age, gender, community and current smoking status (yes/no). Sport fish consumption is not expected to be a major route of exposure to lead since lead levels are low in Ontario sport fish. Other routes of exposure to lead, which are correlated with sport fish consumption, may be involved. However, these results point to the need to consider lead levels in any neurobehavioural/developmental studies of fisheaters and/or their children.

18. Smoking was the primary determinant of cadmium blood levels in this non-occupationally exposed population. Levels were similar to those found in other Canadian studies but considerably higher than those found in a Michigan fisheater study. Consumption of moose/deer meat or organs was not significant in the regression model, but may have played a role in the elevated cadmium level found in one participant.

19. Urinary cotinine was an effective biomarker for smoking status.

20. In this study population, with generally low levels of contaminants, no relationships were found between fish consumption and liver plasma enzymes, thyroid hormones, urinary porphyrins, or d-glucaric acid. Elevations of some of these biomarkers were due to a range of other reasons and required considerable follow-up with participants. Therefore, biomarkers should only be considered when it is clear that the population is exposed and that the biomarker is specific to the exposure of interest. Storing samples for biomarker analysis until contaminant data is available may be a suitable approach (informed consent will usually be required).

21. The results interpretation and communication strategy (communication of individual laboratory results to individual participants) was considerably more detailed than in earlier similar studies. Generating individualized reports for both participants and their physicians was resource intensive but providing detailed information was an ethical responsibility and commitment in this study given public expectations of such

research. Knowing one's own contaminant levels was one of the few benefits of participation and was a clear motivating factor for many participants. It did not generate great anxiety among participants nor inappropriate health care utilization. We can therefore recommend our approach in future research with fish and/or wildlife consumers but counsel researchers to adequately allocate resources.

22. Levels of contaminants (with the exception of cadmium) were generally low compared with other studies of fish and wildlife eaters and below levels known to be associated with adverse health effects. Within these low ranges, PCBs, mercury and lead levels were found to be higher in fish eaters compared with non-eaters. However the magnitude of these differences and the number of people with elevated levels are not sufficient to conduct further health studies with the fish license holder population at this time.

9.0 REFERENCES

Ahlborg UG, Becking GC, Birnbaum LS et al. 1994. Toxic equivalency factors for dioxin-like PCBs. Chemosphere. 28 (6): 1049-1067.

Arias IM, Jakoby WB, Popper H, Schachter D, Shafritz DA. 1988. Biological implications of xenobiotic metabolism. In: *The Liver Biology and Pathology, 2nd ed.* Raven Press, New York. p.355-362.

Armbruster G, Gerow KG, Gutenmann WH, Littman CB, Lisk DJ. 1987. The effects of several methods of fish preparation on residues of polychlorinated biphenyls and sensory characteristics in striped bass. J Food Safety. 8:235-243.

Armbruster G, Gutenmann WH, Lisk DJ. 1988. The effects of six methods of cooking on residues of mercury in striped bass. Nutrition Reports International. 37(1):123-126.

Armbruster G, Gall KL, Gutenmann WH, Lisk DJ. 1989. Effects of trimming and cooking by several methods on poly-chlorinated biphenyl (PCB) residues in bluefish. J Food Safety. 9:235-244.

Asplund L, Svensson B-G, Nilsson A et al. 1993. PCB, p,p'-DDT and p,p'-DDE in human plasma related to fish consumption. In: Svensson B-G. *Human Exposure and Certain Health Implications of Some Toxic and Essential Compounds in Fish.* Thesis, Dept. of Occupational and Environmental Medicine. Lund University, Sweden.

Bahn A, Mills JL, Snyder PJ et al. 1980. Hypothyroidism in workers exposed to polybrominated biphenyls. New England J Med (Jan 3);302(1):31-33.

Benedetti J-L, Dewailly E, Turcotte F, Lefebvre M. 1994. Unusually high blood cadmium associated with cigarette smoking among three subgroups of the general population, Québec, Canada. Sci Total Environ. 152: 161-167.

Berode M, Wietlisbach V, Rickenbach M, Gallemin MP. 1991. Lifestyle and environmental factors as determinants of blood lead levels in a Swiss population. Env Res. 55:1-17.

Borgmann U, Whittle DM. 1991. Contaminant concentration trends in Lake Ontario lake trout (*Salvelinus namaychush*): 1977 to 1988. J Great Lakes Res. 17(3): 368-381.

Brune D, Nordberg GF, Vesterburg O, Gerhardsson L, Wester PO. 1991. A review of normal concentrations of mercury in human blood. The Science of the Total Environment. 100:235-282.

Brown JF, Lawton RW. 1984. Polychlorinated biphenyl (PCB) partitioning between adipose tissue and serum. Bull Environ Contam Toxicol. 33:277-280.

Carrier G, Tremblay CG, Groulx S, Dewailly E. 1990. Polychlorobiphenyl (PCB), polychlorodibenzodioxin (PCDD) and polychlorodibenzofuran (PCDF) exposure of firefighters involved in the PCB fire at St. Basile-le-Grand, Québec, Canada. In: Hutzinger O, Fielder H, eds. *Organohalogen compounds.* Ecoinforma Press, Germany. p.361-368. Seminar, Dioxin 90/EPRI, vol. 3.

Cavan KR, Gilson BL, Riedel D. 1995 (In Press). Intake and dietary importance of freshwater fish in a group of immigrant women from Vietnam. J Cdn Dietetic Assn.

Coad S, Richardson GM. 1995. Consumption of fish and wildlife by native Canadians: estimates based on a review of literature. In prep.

Conacher HBS, Graham, RA, Newsome WH, Graham GF, Verdier P. 1989. The Health Protection Branch total diet program: an overview. Can Inst Food Sci Technol J. 22(4): 322-326.

Connelly NA, Knuth BA, Bisogni CA. 1992. Effects of the Health Advisories and Advisory Changes on Fishing Habits and Fish Consumption in New York Sport Fisheries. Report for New York Sea Grant Institute Project No. R/FHD-2-PD. Series 92-9. Cornell University. Sept 1992. 112 pp.

Cooper GR, Myers GL, Smith SJ, Sampson EJ. 1988. Standardization of lipid, lipoprotein and apolipoprotein measurements. Clin Chem. 34(8B):B95-B109.

Cox C. 1995. Personal communication, March 1995. Ontario Ministry of Environment and Energy, Sport Fish Monitoring Program.

Cox C, Johnson AF. 1990. A study of consumption patterns of Great Lakes salmon and trout anglers. Ontario Ministry of Environment, log 90-2330-901.

Cox C, Vaillancourt A, Johnson AF. 1987. A comparison of the results from the "Guide to eating Ontario sport fish questionnaire. Ontario Min. of Env., Water Resources Branch. Ontario, Canada. 85pp.

Cox C, Vaillancourt A, Johnson AF. 1990. The results of the 1989 "Guide to eating Ontario Sport Fish" questionnaire. Ontario Ministry of Env., Water Resources Branch. Ontario, Canada. 27pp.

Cox C, Vaillancourt A, Hayton A. 1993. The results of the 1992 "Guide to eating Ontario sport fish". Min. of Environment & Energy. Water Resources Branch. Ontario, Canada. 26pp.

Cummings KM, Markello J, Mahoney M, Bhargava AK, McElroy PD, Marshall JR. 1990. Measurement of current exposure to environmental tobacco smoke. Arch Env Health. 45:74-79.

Dar E, Kanarek MS, Anderson HA, Sonzogni WC. 1992. Fish consumption and reproductive outcomes in Green Bay, Wisconsin. Env Res. 59:189-201.

Department of Fisheries & Oceans (DFO). 1989. 1985 Survey of sport fishing in Canada, selected results prepared for the Great Lakes Fishery Commission. Economic and Commercial Analysis Report No. 17. Department of Fisheries & Oceans, Ottawa, Canada. 59pp.

Dewailly E, Laliberté C, Sauvé L, Gingras S, Ayotte P. 1991. La consommations des produits de la mer sur la Basse-côte-nord du golfe St-Laurent: Risques et bénéfices. Centre Hospitalier de l'Université Laval. Décembre, 1991. 150p + appendices.

Dewailly E, Ryan JJ, Laliberté C et al. 1994. Exposure of remote maritime populations to coplanar PCBs. Env Health Pers Supp. 102 (Suppl 1): 205-209.

Ducoffre G, Claeys F and Bruaux P. 1990. Lowering time trend of blood lead levels in Belgium since 1978. Env Res. 51:25-34.

Ebert ES, Price PS, Keenan RE. 1994. Selection of fish consumption estimates for use in the regulatory process. J Exp Anal Env Epi. 4(3): 373-393.

Environment Canada, Dept. of Fisheries and Oceans, Health and Welfare Canada. 1991. Toxic chemicals in the Great Lakes and associated effects. Minister of Supply and Services Canada. Vol 1 & 2. 755 p. Cat. No. En. 37-95/1990-1E.

Fiore BJ, Anderson HA, Hanrahan LP, Olson LJ, Sonzogni WC. 1989. Sport fish consumption and body burden levels of chlorinated hydrocarbons: a study of Wisconsin anglers. Arch Env Health. 44(2):82--88.

Fox GA. 1994. Ed. Bioindicators as a Measure of Success for Virtual Elimination of Persistent Toxic Substances. International Joint Commission. Windsor, Ontario. 37 p.

Fox GA, Kennedy SW, Norstron RJ, Wigfield DC. 1988. Porphyria in herring gulls: a biochemical response to chemical contamination of Great Lakes food chains. Environ Toxicol Chem. 7:831-839.

Goss Gilroy Inc. 1993. Great Lakes Basin Anglers Study: Report on Field Procedures. Contractors report prepared for Great Lakes Health Effects Division, Health and Welfare Canada. 44p + appendices.

Health Canada. 1994. Human health risk assessment for priority substances (Canadian Environmental Protection Act). Minister of Supply and Services Canada. 36pp.

Health and Welfare Canada. 1979. Methylmercury in Canada. Volume 1. Exposure of Indian and Inuit residents to methylmercury in the Canadian environment. Ottawa, Canada. 200p.

Health and Welfare Canada. 1984. Methylmercury in Canada. Volume 2. Exposure of Indian and Inuit residents to methylmercury in the Canadian environment. Ottawa, Canada. 164p.

Health and Welfare Canada. 1989. Canadian Guidelines for Healthy Weights. Report of an expert group convened by Health Promotion Directorate, Health Services and Promotion Branch. Ottawa. 1988. Ministry of Supply and Services Canada Cat. No. H39-134/1989E. 126p.

Health and Welfare Canada. 1991. Proceedings of the workshop on planning phase III studies for the Great Lakes Health Effects Cohort Study. Chronic Diseases in Canada. Supp. 12(6). 24 pp.

Helfand M, Crapo LM. 1990. Screening for thyroid disease. Annals Internal Medicine. 112:840-849

Hovinga M, Sowers M, Humphrey HEB. 1992. Historical changes in serum PCB and DDT levels in an environmentally-exposed cohort. Arch Environ Contam Toxicol. 22:362-366.

Hovinga M, Sowers M, Humphrey HEB. et al. 1993. Environmental exposure and lifestyle predictors of lead, cadmium, PCB and DDT levels in Great Lakes fish eaters. Arch Env Health. 48(2):98-104.

Humphrey HEB. 1983. Evaluation of humans exposed to water-borne chemicals in the Great Lakes. Michigan Dept. of Public Health, Lansing Michigan. 78pp + tables.

Humphrey HEB. 1988. Chemical contaminants in the Great Lakes: the human health aspect. In: Evans M, ed. *Toxic contaminants and ecosystem health: a Great Lakes focus.* John Wiley & Sons. p.153-165.

Hunter J, Maxwell JD, Steward DA, et al. 1972. Increased hepatic microsomal enzyme activity from occupational exposure to certain organochlorine pesticides. Nature. 237:399-401.

International Joint Commission. 1991. Great Lakes Science Advisory Board 1991 Report to the International Joint Commission. International Joint Commission. Windsor, Ont. p.153-165.

Jacobson JL, Humphrey HEB, Jacobson SW, Schwartz SL, Mullin MD, Welch R. 1989. Determinants of polychlorinated biphenyls (PCBs), polybrominated biphenyls (PBBs) and dichlorodiphenyl trichloroethane (DDT) levels in the sera of young children. Am J Public Health. 79(10):1401-1404.

Jacobson SW, Jacobson JL, Schwartz P, Fein G. 1983. Intrauterine exposure of human newborns to PCBs: measures of exposure. In: D'Itri, FM, Kamrin M, eds. *PCBs: human and environmental hazards*. Boston: Butterworth. p. 311-333.

Jarvis MJ, Turnstall-Pedoe H, Feyerband C, Vesey C, Salojee Y. 1987. Comparisons of tests used to distinguish smokers and non-smokers. Am J Public Health. 77 (100):1435-1438.

Jensen AA. 1991. Transfer of chemical contaminants into human milk. In: Jensen AA, Slorach SA, eds. *Chemical contaminants in human milk*. CRC Press, Inc. p.9-19.

Kinloch D, Kuhnlein H, Muir DCG. 1992. Inuit foods and diet: a preliminary assessment of benefits and risks. The Science of the Total Envt. 122:247-278

Kreiss K. 1985. Studies on populations exposed to polychlorinated biphenyls. Env Health Persp. 60:193-199.

Kreiss K, Zack MM, Kimbrough RD, Needham LL, Smrek AL, Jones BT. 1981. Association of blood pressure and polychlorinated biphenyl levels. JAMA. 245(24):2505-2509.

Kutz FW, Barnes DG, Bottimore DF, Greim H, Bretthauer EW. 1990. The International Toxicity Equivalency Factor (I-TEF) method of risk assessment for complex mixtures of dioxins and related compounds. Chemosphere. 20(7-9):751-759.

Laliberté C, Dewailly E, Gingras S et al. 1992. Mercury contamination in fishermen of the Lower North Shore of the Gulf of St. Lawrence (Québec, Canada). In: Vernet J-P, ed. *Impact of Heavy Metals on the Environment. Trace Metals in the Environment Vol 2.* Elsevier, Amsterdam, p. 15-28.

Lauwerys RR, Hoet P. 1993. Industrial chemical exposure, guidelines for biological monitoring. Lewis Publishers. Boca Raton, Fl. 318pp.

Mah S. 1990. Summary of blood lead levels in Alberta. Communication from Alberta Occupational Health and Safety. Cited in: *Working Group on Blood Lead Intervention Levels and Strategies*. 1995 (in press).

Market Facts of Canada. 1991. National Seafood Consumption study. Prepared for Health and Welfare Canada. Toronto, Ontario. 26pp + tables.

Nieboer E, Jusys JA, Julian JA et al. 1988. Biological monitoring of Ontario cadmium workers: determinants of whole blood and urinary cadmium levels. Poster, Intl. Meeting on Molecular Mechanism of Metal Toxicity & Carcinogenicity, Collzio Universitario, Urbina, Italy. Sept. 19-22.

Notten WRF, Henderson PT. 1977. The interaction of chemical compounds with the functional state of the liver. Int Arch Occup Environ Hlth., 38:209-220.

Ontario Ministry of the Environment (MOE), 1991. Guide to Eating Ontario Sport Fish, Fifteenth Edition, Queen's Printer for Ontario. Toronto, Ontario.

Perrault A. 1992. Trends in exposure to methylmercury among the Cree of James Bay (1975-1988). Cree Board of Health and Social Services of James Bay. 40pp + tables, apps.

Phillips DL, Pirkle JL, Burse VW, Bernert JT, Henderson LO, Needham LL. 1989.

Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. Arch Environ Contam Toxicol. 18:495-500.

Poon R, Villeneuve DC, Chu I, Kinach R. 1993. HPLC determination of d-glucaric acid in human urine. J of Anal Toxicol. 17:146-150.

Richardson GM, Currie DJ. 1993. Estimating fish consumption rates for Ontario Amerindians. J Exp Anal Environ Epi. 3(1):23-38.

Ryan JJ, Lau BP-Y, Boyle MJ. 1994. Dioxin-like compounds in human blood. In: Matsuo T, Caprioli RM, Gross ML, Seyama Y, eds. In: *Biological Mass Spectrometry: Present and Future*. John Wiley & Sons Ltd. pp. 583-602.

Santé Québec. 1994. A health profile of the Inuit. Report of the Santé Québec Health Survey among the Inuit of Nunavik, 1992. Volume 1: Health determining factors. Montreal, Québec. 252pp + apps.

SAS Inst. Inc. 1988. SAS/STAT User's Guide, Release 6.03 Edition. 1028pp.

Savoie PJ and Bowlby PJ. 1990. Estimates of the total angling harvest in the Canadian waters of Lake Ontario in 1989. In: Ontario Ministry of Natural Resources. *Lake Ontario Fisheries Unit, 1990 Annual Report LOA 91.1*. Chapter 8.

Schwartz PM, Jacobson SW, Fein G, Jacobson JL, Price HA. 1983. Lake Michigan fish consumption as a source of polychlorinated biphenyls in human cord serum, maternal serum and milk. Am J Public Health. 73 (3):293-296.

Sepkovic D, Haley NJ. 1985. Biomedical applications of cotinine quantitation in smoking related research. Am J Public Health. 75:663-665.

Skea JC, Simonin HA, Harris EJ, et al. 1979. Reducing levels of mirex, Aroclor 1254, and DDE by trimming and cooking Lake Ontario brown trout (Salmo trutta LINNAEUS) and smallmouth bass (Micropterus dolomieui Lacepede). J Great Lakes Res. 5(3):153-159.

Sonzogni W, Maack L, Gibson T, Degenhardt D, Anderson H, Fiore B. 1991. Polychlorinated biphenyl congeners in blood of Wisconsin sport fish consumers. Arch Env Contam Toxicol. 20:56-60.

SPR Ass. Inc. 1991. Report on the telephone survey phase and conclusion of the Great Lakes Basin Anglers Pilot Survey. Prepared for Health and Welfare Canada. 22p + appendices.

St. Lawrence RAP Team. 1992. Remedial action plan for the Cornwall-Lake St. Francis Area. Stage 1 Report: Environmental Conditions and Problem Definitions. August 1992. ISBN:0-662-19958-8.

Stachiw NC, Zabik ME, Booren AM, Zabik MJ. 1988. Tetrachlorodibenzo-p-dioxin residue reduction through cooking/processing of restructured carp fillets. J Agric Food Chem. 36:848-852.

Steward R, Olson J. 1991. Toxicology and environmental chemistry of exposure to toxic chemicals. In: Flint RW, Vena J, eds. *Human Health Risks from Chemical Exposure: The Great Lakes Ecosystem*. Lewis Publishers Inc. Chelsea, MI. pp. 27-60.

Stewart TJ, Savoie PJ, Bowlby, JN. 1989. Salmonoid angler survey of the western and central basins of Lake Ontario, 1989. In: Lake Ontario Fisheries Unit. *1989 Annual Report, LOA 90.1*. Ontario Ministry of Natural Resources. Chapter 12.

Strik, JJTWA. 1981. Health status of factory workers with long-term exposure to chlorinated hydrocarbons, In: Khan MAQ, Stanton RH, eds. *Toxicology of Halogenated Hydrocarbons: Health and Ecological Effects*. Pergamon Press, New York. pp. 66-69.

Svensson B-G, Nilsson A, Hansson H, Rappe C, Akesson B, Skerfvings. 1991. Exposure to dioxins and dibenzofurans through the consumption of fish. New Eng J Med. 324:8-12.

Subcommittee of reproductive and neurodevelopmental toxicology, Committee on Biologic markers. 1989. Biomarkers in reproductive toxicology. National Academic Press. Washington, DC.

Thompson SR, Stone R, Nanchahal K, Wald NJ. 1990. Relation of urinary of cotinine concentrations to cigarette smoking and to exposure to other people's smoke. Thorax. 45(5):356-361.

Todd JW, Durkee J. 1989. The Great Lakes Basin: a regional focus on the environment and human health. Conference proceedings. Great Lakes Coalition of Public Health Associations and Illinois Public Health Association. Springfield, Ill.

VanOostdam J, Kagey B. 1991. Epidemiology of exposure to toxic chemicals. In: Flint RW, Vena J, eds. *Human Health Risks from Chemical Exposure: The Great Lakes Ecosystem*. Lewis Publishers Inc. Chelsea, MI. pp. 27-60.

Vine MF, Hulka BS, Margolin BH et al. 1993. Cotinine concentrations in semen, urine and blood of smokers and nonsmokers. Am J Public Health. 83:1335-1338.

Voiland M, Gall KL, Lisk DJ, MacNeil DB. 1991. Effectiveness of recommended fat-trimming procedures on the reduction of PCB and mirex levels in brown trout (Salmo trutta) from Lake Ontario. J. Great Lakes Res. 17(4): 454-460.

Wall M, Johnson J, Jacob P, Benowitz N. 1988. Cotinine in the serum, saliva and urine of nonsmokers, passive smokers and active smokers. Am J Public Health. 78(6):699-701.

Watanabe T, Kasahara M, Nakatsuka H, Ikeda M. 1987. Cadmium and lead contents of cigarettes produced in various areas of the world. The Science of the Total Environment. 66:29-37.

Willers S, Attewell R, Bensryd I, Schutz A, Skarping G, Vanter M. 1992. Exposure to environmental tobacco smoke in the household and urinary cotinine excretion, heavy metals retention, and lung function. Arch Env Health. 47(5):357-363.

Williams D, LeBel GL, Junkins E. 1988. Organohalogen residues in human adipose autopsy samples from six Ontario municipalities. J Assoc of Anal Chem. 7(2):410-414

Working Group on Blood Lead Intervention Levels and Strategies. 1995 (in press). Update of evidence for low level health effects of lead and proposed blood lead intervention levels and strategies. Federal-Provincial Committee on Environmental and Occupational Health. Health Canada, Ottawa.

Zabik ME, Hoojjat P, Weaver CM. 1979. Polychlorinated biphenyls, dieldrin and DDT in lake trout cooked by broiling, roasting or microwaving. Bull Environ Contam Toxicol. 21:136-143.

Zabik ME, Merrill C, Zabik MJ. 1982. PCBs and other xenobiotics in raw and cooked carp. Bull Environ Contam Toxicol. 28:710-715.

Zabik ME, Zabik MJ, Humphrey H. 1993. Assessment of contaminants in five species of Great Lakes fish at the dinner table. Final Report Part I. Fish procurement, processing, pesticides and total PCBs, polynuclear aromatic hydrocarbons. March 1, 1993. Michigan State University and Michigan Dept. of Health.

Appendix 1
Great Lakes Basin Anglers Survey
(mail-out)

Health and Welfare Canada   Santé et Bien-être social Canada

Great Lakes Basin Anglers Survey
An Environmental Health Study

(Confidential when completed)

Instructions
This questionnaire is divided into two parts. Please complete Section A. If you are married or living as married please ask your spouse to complete Section B (page 11). Most of the questions require that you indicate your answer by checking the box next to the appropriate answer. For other questions, please read the instructions with the question. Please do not write in shaded areas. When the questionnaire is completed, please return it in the postage-paid envelope provided.

1. Is the information on the address label correct?
1 ○ Yes
2 ○ No. Please indicate any necessary corrections below.

Name

Address

City/Town   Province   Postal Code

Great Lakes Health Effects Program

Canada

NHW-HPB-04095
PIB P-PU-076

Section A:
To Be Completed by You

Note: It is important that everyone who receives the questionnaire complete and return it regardless of whether you eat fish or not.

2. We would like to get an idea of the number and kind of fish that were caught and kept for eating during the last 12 months by **all members of your household,** and the location where these fish were caught. For each location indicate how many of each species were **caught and kept for eating** by all members of your household. You may refer to the attached **map** for assistance in locating fishing areas.

Did you or any members of your household catch any fish in the last 12 months?

1 ○ No. → Go to Question **3.**
2 ○ Yes

a. Were any fish caught and kept for eating from the **St. Lawrence River**?

1 ○ No. → Go to **b.**
2 ○ Yes. How many of the following?

		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Maskinonge (muskie)	
6	White bass	
7	Small and large-mouthed bass	
8	Panfish (bluegill, sunfish, crappie, rock bass)	
9	Carp	
10	Catfish and bullheads	
11	Other (specify)	
	☐☐☐	
	☐☐☐	

b. Were any fish caught and kept for eating from **Lake Ontario**?

1 ○ No. → Go to **c.**
2 ○ Yes. How many of the following?

		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Rainbow trout	
6	Other trout	
7	Maskinonge (muskie)	
8	White bass	
9	Small and large-mouthed bass	
10	Whitefish	
11	Salmon, any species	
12	Smelt (*number of buckets*)	
13	Panfish (bluegill, sunfish, crappie, rock bass)	
14	Carp	
15	Catfish and bullheads	
16	Eels	
17	Other (specify)	
	☐☐	
	☐☐	

c. Were any fish caught and kept for eating from the **Niagara River**?

1 ○ No. → Go to **d.**
2 ○ Yes. How many of the following?

		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Rainbow trout	
6	Other trout	
7	Maskinonge (muskie)	
8	White bass	
9	Small and large-mouthed bass	
10	Salmon, any species	
11	Smelt (*number of buckets*)	
12	Panfish (bluegill, sunfish, crappie, rock bass)	
13	Freshwater drum	
14	Other (specify)	
	☐☐☐	
	☐☐☐	

d. Were any fish caught and kept for eating from **Lake Erie**?

1 ○ No. → Go to **e.**
2 ○ Yes. How many of the following?

		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Rainbow trout	
6	Maskinonge (muskie)	
7	White bass	
8	Small and large-mouthed bass	
9	Whitefish	
10	Salmon, any species	
11	Smelt (*number of buckets*)	
12	Panfish (bluegill, sunfish, crappie, rock bass)	
13	Carp	
14	Catfish and bullheads	
15	Freshwater drum	
16	Other (specify)	
	☐☐☐	
	☐☐☐	

e. Were any fish caught and kept for eating from the **Detroit River, Lake St. Clair, or St. Clair River**?

1 ○ No. → Go to **f.**
2 ○ Yes. How many of the following?

		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Rainbow trout	
6	Other trout	
7	White bass	
8	Small and large-mouthed bass	
9	Salmon, any species	
10	Smelt (*number of buckets*)	
11	Panfish (bluegill, sunfish, crappie, rock bass)	
12	Catfish and bullheads	
13	Freshwater drum	
14	Other (specify)	
	☐☐☐	
	☐☐☐	

f. Were any fish caught and kept for eating from the **Lake Huron, Georgian Bay, St. Mary's River**?

1 ○ No. → Go to **g.**
2 ○ Yes. How many of the following?

		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Rainbow trout	
6	Other trout	
7	Maskinonge (muskie)	
8	White bass	
9	Small and large-mouthed bass	
10	Whitefish	
11	Salmon, any species	
12	Smelt (*number of buckets*)	
13	Panfish (bluegill, sunfish, crappie, rock bass)	
14	Carp	
15	Catfish and bullheads	
16	Freshwater drum	
17	Lake sturgeon	
18	Sucker	
19	Herring	
20	Burbot	
21	Other (specify)	
	☐☐☐	
	☐☐☐	

g. Were any fish caught and kept for eating from **Lake Superior**?

1 ○ No. → Go to **h.**
2 ○ Yes. How many of the following?

		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Rainbow trout	
6	Other trout	
7	Small and large-mouthed bass	
8	Whitefish	
9	Salmon, any species	
10	Smelt (*number of buckets*)	
11	Catfish and bullheads	
12	Other (specify)	
	☐☐☐	
	☐☐☐	

h. Were any fish caught and kept for eating from the **inland waters of Northern Ontario**?

1 ○ No. → Go to **i.**
2 ○ Yes. How many of the following?

		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Other trout	
6	Maskinonge (muskie)	
7	Small and large-mouthed bass	
8	Whitefish	
9	Panfish (bluegill, sunfish, crappie, rock bass)	
10	Carp	
11	Catfish and bullheads	
12	Lake sturgeon	
13	Burbot	
14	Other (specify)	
	☐☐☐	
	☐☐☐	

i. Were any fish caught and kept for eating from the **inland waters of Central and Southern Ontario**?

1 ○ No. → Go to **j.**
2 ○ Yes. How many of the following?

		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Rainbow trout	
6	Other trout	
7	Maskinonge (muskie)	
8	Small and large-mouthed bass	
9	Whitefish	
10	Salmon, any species	
11	Smelt (*number of buckets*)	
12	Panfish (bluegill, sunfish, crappie, rock bass)	
13	Carp	
14	Catfish and bullheads	
15	Eels	
16	Herring	
17	Burbot	
18	Other (specify)	
	☐☐☐	
	☐☐☐	

j. Were any fish caught and kept for eating from the **inland waters of Eastern Ontario**?

1 ○ No. → Go to **k.**
2 ○ Yes. How many of the following?

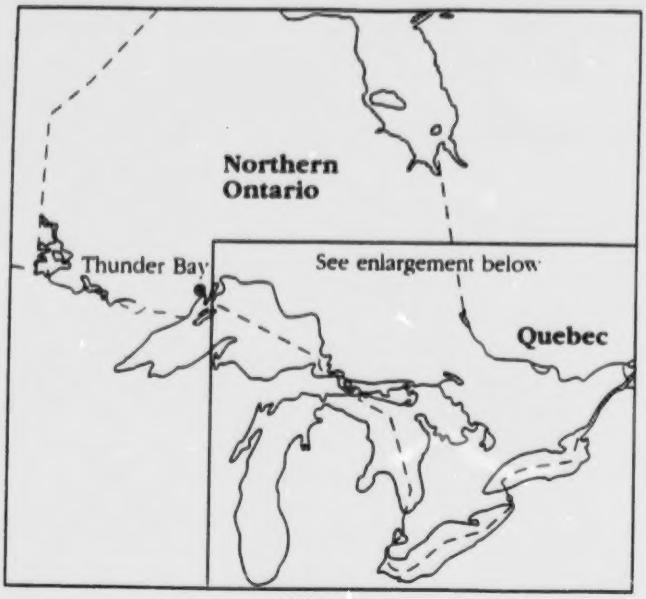
		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Maskinonge (muskie)	
6	Small and large-mouthed bass	
7	Panfish (bluegill, sunfish, crappie, rock bass)	
8	Carp	
9	Catfish and bullheads	
10	Eels	
11	Sucker	
12	Burbot	
13	Other (specify)	
	☐☐☐	
	☐☐☐	

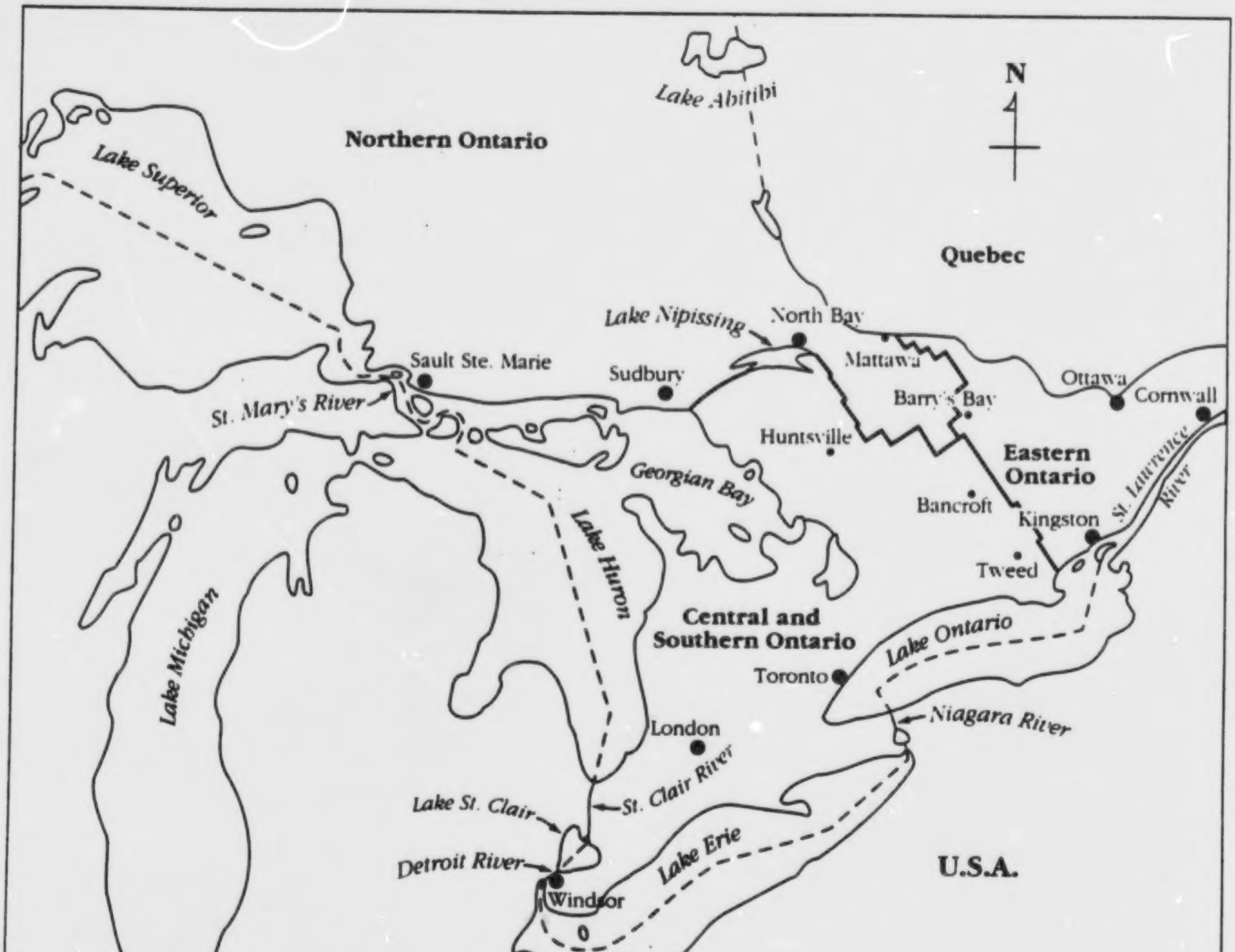
k. Were any fish caught and kept for eating from **any locations outside Ontario**?

1 ○ No. → Go to Question **3.**
2 ○ Yes. How many of the following?

		Number of fish in last 12 months
1	Walleye and sauger (pickerel)	
2	Pike	
3	Perch	
4	Lake trout	
5	Rainbow trout	
6	Other trout	
7	Maskinonge (muskie)	
8	Small and large-mouthed bass	
9	Whitefish	
10	Salmon, any species	
11	Smelt (*number of buckets*)	
12	Panfish (bluegill, sunfish, crappie, rock bass)	
13	Carp	
14	Catfish and bullheads	
15	Freshwater drum	
16	Lake sturgeon	
17	White bass	
18	Sucker	
19	Eels	
20	Herring	
21	Burbot	
22	Other (specify)	
	☐☐☐	
	☐☐☐	

Ontario Fishing Areas


Northern Ontario
Thunder Bay
See enlargement below
Quebec


Lake Abitibi
N
Northern Ontario
Lake Superior
Quebec
Lake Nipissing
North Bay
Mattawa
Sault Ste. Marie
Sudbury
Ottawa
Cornwall
St. Mary's River
Barry's Bay
Huntsville
Eastern Ontario
Georgian Bay
Bancroft
Kingston
St. Lawrence River
Tweed
Lake Huron
Lake Michigan
Central and Southern Ontario
Lake Ontario
Toronto
Niagara River
London
St. Clair River
Lake St. Clair
Detroit River
Lake Erie
Windsor
U.S.A.

Your Freshwater Fish and Wildlife Consumption

3. During the past 12 months did you **eat** any sport-caught fish (fish caught by you, family members or friends)?

1 ○ No. → Go to Question **4.**
2 ○ Yes. → Go to Question **5.**

4. Have you **ever** eaten sport-caught fish?

1 ○ No. → Go to Question **7.**
2 ○ Yes.

When did you stop **eating** sport-caught fish?

19_____.

Approximately how many years did you eat sport-caught fish?

_____ years. → Go to Question **7.**

5. Approximately how many years have you been eating sport-caught fish?

_____ years.

6. During **the past 12 months,** how many times did you eat sport-caught fish? (Include any sportfish caught by you, family members or friends.) (Check **one** only.)

1 ○ On vacation only (_____ times)
2 ○ Daily
3 ○ More than once a week
4 ○ Once a week
5 ○ Once every 2 weeks
6 ○ Once a month
7 ○ _____ times in the last 12 months

7. During **the past 12 months,** how many times did you eat freshwater fish purchased in a store, from an individual, or from a restaurant? (Check **one** only.)

1 ○ None
2 ○ Daily
3 ○ More than once a week
4 ○ Once a week
5 ○ Once every 2 weeks
6 ○ Once a month
7 ○ _____ times in the last 12 months

8. Are you aware of, or do you have a current copy of the "Guide to Eating Ontario Sportfish" published by the Ontario Ministries of Environment and Natural Resources? (Check **one** only.)

1 ○ No, I am not aware of the "Guide". → Go to Question **10.**
2 ○ Yes, I am aware of the "Guide" and have a current copy.
3 ○ Yes, I am aware of the "Guide" but I do **not** have a current copy.

9. Do you follow the fish consumption advice provided in the "Guide to Eating Ontario Sportfish"? (Check **one** only.)

1 ○ Never
2 ○ Occasionally
3 ○ Usually
4 ○ Always

10. Have you eaten any wildfowl or game in the last 12 months?

1 ○ No. → Go to Question **11.**
2 ○ Yes. Which of the following wildfowl or game have you eaten in the last 12 months? (Check all that apply.)

1 ○ Wild fish-eating ducks (mergansers)
2 ○ Any other wild ducks or geese
3 ○ Any wild game bird other than ducks or geese
4 ○ Turtle, any species
5 ○ Rabbit
6 ○ Bear
7 ○ Moose
8 ○ Deer
9 ○ Other (please specify)

____
____

11. What sex are you?

1 ○ Male
2 ○ Female

12. During your lifetime, how many years have you lived in Ontario?

_____ years

13. How many years have you lived in the community where you are now living?

_____ years

14. What is the highest level of education that you have achieved? (Check **one** only.)

1 ○ Any of grades 1 to 8
2 ○ Any of grades 9 to 11
3 ○ High school graduate (grade 12 or 13)
4 ○ Started college or university but did not complete
5 ○ Post-secondary diploma or degree (college, trade school, university)

15. What is your current employment status? (Check all that apply.)

1 ○ Employed part time
2 ○ Employed full time
3 ○ Unemployed
4 ○ Homemaker
5 ○ Disabled
6 ○ Retired
7 ○ Student

16. We would like some information on your present or most recent job. If you are (or were) working at more than one job please describe the job where you work (or worked) the most hours.

a. What kind of business, industry or service do (or did) you work for? (Give a full description. For example, dairy farm, high school, insurance agency, rubber manufacturing plant. Please print.)

____
____

b. What kind of work do (or did) you do in your job? (For example, dairy farmer, english teacher, accountant, assembly line worker. Please print.)

____
____

c. How long have you been working (or did you work) at this job?

_____ years OR _____ months, if less than 1 year.

d. Are you still working at this job?

1 ○ No. → When did you stop? 19 _____.
2 ○ Yes

17. In your living or working environment, have you come into contact with any of the following materials over the past 12 months? (Check **one** circle for each of **a** to **i.**)

a. Dust from wood, grain, hay, or straw
1 ○ Never 2 ○ Occasionally 3 ○ Often

b. Dust from silica, granite or rock dust
1 ○ Never 2 ○ Occasionally 3 ○ Often

c. Glass fibre dust or asbestos
1 ○ Never 2 ○ Occasionally 3 ○ Often

d. Dust or fumes from lead, cadmium, nickel, chromium or mercury
1 ○ Never 2 ○ Occasionally 3 ○ Often

e. Fumes from solvents, paints, or gasoline
1 ○ Never 2 ○ Occasionally 3 ○ Often

f. Resins or isocyanates
1 ○ Never 2 ○ Occasionally 3 ○ Often

g. Pesticides (bug or weed killers)
1 ○ Never 2 ○ Occasionally 3 ○ Often

h. Coal tar or pitch
1 ○ Never 2 ○ Occasionally 3 ○ Often

i. Other (please specify): ☐☐☐
____
2 ○ Occasionally 3 ○ Often

18. During the past year, approximately how much beer, wine, or hard liquor (whiskey, vodka, gin, rum, brandy, liqueurs, etc.) have you had during an average week? (Enter "0" if you had less than one drink per week of that beverage.)

____ cans or bottles of beer per week.
____ glasses of wine per week.
____ drinks of liquor per week.

19. Do you smoke regularly (one or more cigarettes, cigars or pipes per day)?
1 ○ No
2 ○ Yes

20. What was your approximate total household income for the year 1990 before income tax deductions? (Check **one** only.)
1 ○ Less than $30,000
2 ○ $30,000–$59,999
3 ○ $60,000 or more

21. How tall are you?
____ feet ____ inches OR ____ metres

22. How much do you weigh?
____ pounds OR ____ kilograms

23. Have you ever been told **by a doctor** that you had any of the following? (Check all that apply; if you have not had any of the following then please check "None of the above".)

1 ○ High blood pressure or hypertension
2 ○ Kidney disease
3 ○ Heart disease
4 ○ Liver disease
5 ○ Diabetes
6 ○ Asthma
7 ○ Migraine headaches
8 ○ Stomach ulcer
9 ○ Bowel disease
10 ○ Infertility
11 ○ Hay fever or other allergies
12 ○ Miscarriage
13 ○ Goiter or thyroid problems
14 ○ Arthritis or rheumatism
15 ○ Skin allergies or other skin diseases
16 ○ Emphysema, chronic bronchitis or persistent cough
17 ○ Epilepsy or seizures
18 ○ Neurological disorders (other than epilepsy)
19 ○ Cancer; what type? ☐☐☐
____
20 ○ **None of the above**

24. Has **a doctor** ever said that any children (**under age 18**) currently living in your household had any of the following? (Check all that apply; if they have not had any of the following then please check "None of the above".)

1 ○ **No children in the household.** → Go to Question **25.**
2 ○ Persistent ear infections
3 ○ Kidney disease
4 ○ Liver disease
5 ○ Diabetes
6 ○ Asthma
7 ○ Migraine headaches
8 ○ Hay fever or other allergies
9 ○ Bowel disease
10 ○ Arthritis or rheumatism
11 ○ Goiter or thyroid problems
12 ○ Chronic bronchitis or persistent cough
13 ○ Epilepsy or seizures
14 ○ Neurological disorders (other than epilepsy)
15 ○ Skin allergies or other skin diseases
16 ○ Birth defect of any kind; what type? ☐☐☐
____
17 ○ Cancer; what type? ☐☐☐
____
18 ○ **None of the above**

25. For each person currently living in your household, please list their name, date of birth, sex, and place of birth. Start with yourself, your spouse if applicable, and then all other members of your household. Please print.

	First name	Initial	Surname	Date of birth Day	Month	Year	Sex (circle M or F)	Province of birth OR country if not Canada
Yourself								
1.							M F	
Your spouse								
2.							M F	
Others								
3.							M F	
4.							M F	
5.							M F	
6.							M F	
7.							M F	
8.							M F	
9.							M F	

Please make sure all household members currently living in your household have been listed above, including yourself.

26. In case you move and Health and Welfare Canada researchers need to contact you, please provide the name, address and telephone number of an individual who would know your new address.

Name

Street

City/Town

Province

Postal Code

Telephone (_ _ _) _ _ _-_ _ _ _

27. Please provide your home telephone number in case there is a need to clarify any of the information supplied above.

(_ _ _) _ _ _-_ _ _ _

Thank you for your cooperation in completing this questionnaire.

If you are married or living as married please ask your spouse to complete section B.

If you have any comments please use the space provided on page 14. Please return the questionnaire using the postage-paid envelope provided.

Section B: Questions for Your Spouse

Freshwater Fish and Wildlife Consumption

28. During the past 12 months did you **eat** any sport-caught fish (fish caught by you, family members or friends)?

1 ○ No. → Go to Question **29.**
2 ○ Yes. → Go to Question **30.**

29. Have you **ever** eaten sport-caught fish?

1 ○ No. → Go to Question **32.**
2 ○ Yes.

When did you stop **eating** sport-caught fish?

19_____.

Approximately how many years did you eat sport-caught fish?

_____ years. → Go to Question **32.**

30. Approximately how many years have you been eating sport-caught fish?

_____ years.

31. During **the past 12 months,** how many times did you eat sport-caught fish? (Include any sportfish caught by you, family members or friends.) (Check **one** only.)

1 ○ On vacation only (_____ times)
2 ○ Daily
3 ○ More than once a week
4 ○ Once a week
5 ○ Once every 2 weeks
6 ○ Once a month
7 ○ _____ times in the last 12 months

32. During **the past 12 months,** how many times did you eat freshwater fish purchased in a store, from an individual, or from a restaurant? (Check **one** only.)

1 ○ None
2 ○ Daily
3 ○ More than once a week
4 ○ Once a week
5 ○ Once every 2 weeks
6 ○ Once a month
7 ○ _____ times in the last 12 months

33. Are you aware of, or do you have a current copy of the "Guide to Eating Ontario Sportfish" published by the Ontario Ministries of Environment and Natural Resources? (Check **one** only.)

1 ○ No. I am not aware of the "Guide". → Go to Question **35.**
2 ○ Yes. I am aware of the "Guide" and have a current copy.
3 ○ Yes. I am aware of the "Guide" but I do **not** have a current copy.

34. Do you follow the fish consumption advice provided in the "Guide to Eating Ontario Sportfish"? (Check **one** only.)

1 ○ Never
2 ○ Occasionally
3 ○ Usually
4 ○ Always

35. Have you eaten any wildfowl or game in the last 12 months?

1 ○ No. → Go to Question **36.**
2 ○ Yes. Which of the following wildfowl or game have you eaten in the last 12 months? (Check all that apply.)

1 ○ Wild fish-eating ducks (mergansers)
2 ○ Any other wild ducks or geese
3 ○ Any wild game bird other than ducks or geese
4 ○ Turtle, any species
5 ○ Rabbit
6 ○ Bear
7 ○ Moose
8 ○ Deer
9 ○ Other (please specify)

____

____

36. What sex are you?

1 ○ Male

2 ○ Female

37. During your lifetime how many years have you lived in Ontario?

_____ years

38. How many years have you lived in the community where you are now living?

_____ years

39. What is the highest level of education that you have achieved? (Check **one** only.)

1 ○ Any of grades 1 to 8

2 ○ Any of grades 9 to 11

3 ○ High school graduate (grade 12 or 13)

4 ○ Started college or university but did not complete

5 ○ Post-secondary diploma or degree (college, trade school, university)

40. What is your current employment status? (Check all that apply.)

1 ○ Employed part time

2 ○ Employed full time

3 ○ Unemployed

4 ○ Homemaker

5 ○ Disabled

6 ○ Retired

7 ○ Student

41. We would like some information on your present or most recent job. If you are (or were) working at more than one job please describe the job where you work (or worked) the most hours.

a. What kind of business, industry or service do (or did) you work for? (Give a full description. For example, dairy farm, high school, insurance agency, rubber manufacturing plant. Please print.)

_____________________________ ☐☐☐

_____________________________ ☐☐☐

b. What kind of work do (or did) you do in your job? (For example, dairy farmer, english teacher, accountant, assembly line worker. Please print.)

_____________________________ ☐☐☐

_____________________________ ☐☐☐

c. How long have you been working (or did you work) at this job?

_____ years OR _____ months, if less than 1 year.

d. Are you still working at this job?

1 ○ No. → When did you stop? 19 _____.

2 ○ Yes

42. In your living or working environment, have you come into contact with any of the following materials over the past 12 months? (Check **one** circle for each of **a** to **i.**)

a. Dust from wood, grain, hay, or straw
1 ○ Never 2 ○ Occasionally 3 ○ Often

b. Dust from silica, granite or rock dust
1 ○ Never 2 ○ Occasionally 3 ○ Often

c. Glass fibre dust or asbestos
1 ○ Never 2 ○ Occasionally 3 ○ Often

d. Dust or fumes from lead, cadmium, nickel, chromium or mercury
1 ○ Never 2 ○ Occasionally 3 ○ Often

e. Fumes from solvents, paints, or gasoline
1 ○ Never 2 ○ Occasionally 3 ○ Often

f. Resins or isocyanates
1 ○ Never 2 ○ Occasionally 3 ○ Often

g. Pesticides (bug or weed killers)
1 ○ Never 2 ○ Occasionally 3 ○ Often

h. Coal tar or pitch
1 ○ Never 2 ○ Occasionally 3 ○ Often

i. Other (please specify):
______________________ ☐☐☐
2 ○ Occasionally 3 ○ Often

43. During the past year, approximately how much beer, wine, or hard liquor (whiskey, vodka, gin, rum, brandy, liqueurs, etc.) have you had during an average week? (Enter "0" if you had less than one drink per week of that beverage.)

_____ cans or bottles of beer per week.
_____ glasses of wine per week.
_____ drinks of liquor per week.

44. Do you smoke regularly (one or more cigarettes, cigars or pipes per day)?
1 ○ No
2 ○ Yes

45. How tall are you?
_____ feet _____ inches OR _____ metres

46. How much do you weigh?
_____ pounds OR _____ kilograms

47. Have you ever been told **by a doctor** that you had any of the following? (Check all that apply; if you have not had any of the following then please check "None of the above".)

1 ○ High blood pressure or hypertension
2 ○ Kidney disease
3 ○ Heart disease
4 ○ Liver disease
5 ○ Diabetes
6 ○ Asthma
7 ○ Migraine headaches
8 ○ Stomach ulcer
9 ○ Bowel disease
10 ○ Infertility
11 ○ Hay fever or other allergies
12 ○ Miscarriage
13 ○ Goiter or thyroid problems
14 ○ Arthritis or rheumatism
15 ○ Skin allergies or other skin diseases
16 ○ Emphysema, chronic bronchitis or persistent cough
17 ○ Epilepsy or seizures
18 ○ Neurological disorders (other than epilepsy)
19 ○ Cancer: what type?
______________________ ☐☐☐
20 ○ **None of the above**

Thank you for your cooperation in completing this questionnaire.

If you have any comments please use the space provided on page 14.

Please return the questionnaire in the postage-paid envelope provided.

Appendix 2
Consent form

Informed Consent Form
Great Lakes Basin Anglers' Study
Great Lakes Health Effects Program
Health and Welfare Canada

If you are interested in participating in the study and you agree with the following specific provisions please sign and return one copy of this form, using the postage paid envelope provided, to the address given at the bottom of this form. You may keep the second copy for your records.

Specific Provisions

1. I am willing to participate in the study, including:
   1) completion of a detailed dietary questionnaire regarding freshwater fish consumption habits and
   2) investigation of the levels of contaminants and biomarkers in my blood, urine and hair samples.

2. I have been made aware of any potential risks and discomforts associated with these procedures. These include discomfort and swelling or bruising due to blood being drawn from the arm. There are no other risks of which I am aware.

3. I know that I can ask any questions concerning the procedures or other aspects of this study.

4. I have had an explanation of all medical terminology in connection with this study.

5. I understand that my participation in this study is voluntary. I also understand that I may withdraw this consent and stop my participation at any time by informing Goss Gilroy Inc. (1-800-463-8236) of my withdrawal.

6. I understand that Health and Welfare Canada will maintain the confidentiality of participants by identifying them with a number. This means that my name or confidential information about me will not be made public.

7. I understand that if I have future questions about the research or about research subject's rights, I should contact Goss Gilroy Inc. (1-800-463-8236) or Jill Kearney, Health and Welfare Canada, telephone (613) 957-8487.

8. I understand that I will be sent a summary of my results in the mail. If any contaminants levels or other measurements in my blood, hair and urine samples are of concern, I would like to *(please check)*:

   ___ discuss the results with the medical advisor to the study.

   ___ have the results of my laboratory analyses sent directly to my own doctor.

   ___ I also give permission for the medical advisor and my own doctor to discuss the results if necessary.

   The name and address of my doctor is:

   Dr. ______________________________
   Address: ______________________________
   ____
   ____
   Phone: __________

I have read the specific provisions of this informed consent form and the information has been presented to me in a satisfactory manner.

Name: ______________
Address: ______________
____
Phone: ______________

Signature of Participant: ______________ Date: ________

Signature of Witness: ______________ Date: ________

Please mail this form, using the envelope provided to:

Goss Gilroy Inc.
Suite 900
150 Metcalfe St.
Ottawa, Ontario
K2P 1P1

Informed Consent Form
Great Lakes Basin Anglers' Study
Health and Welfare Canada

Information Sheet

General information

The Great Lakes Health Effects Program of Health and Welfare Canada is carrying out a research study to look at people who eat sport fish and their exposure to environmental pollutants such as PCB's, pesticides and mercury. We are looking for participants who eat fish from the Great Lakes or St. Lawrence River and participants who don't eat any freshwater fish. Non-consumers will be used to provide information that can be compared to people who do eat sportfish. Your participation may help us understand how humans are exposed to environmental pollutants. Some participants may benefit through assistance in reducing their exposure.

You could help in this effort by answering a questionnaire and providing blood, hair and urine samples. We will provide you with a plastic container to collect the first urine sample of the morning. Our staff will visit your home at a convenient, pre-arranged time to get the urine specimen, administer the questionnaire, and collect a blood and hair sample. The visit should last approximately one hour.

The study is being conducted by the Great Lakes Health Effects Program, Health and Welfare Canada. Ms. Jill Kearney is the project co-ordinator in this study. Dr. Donald Cole is the medical advisor to the study. Goss Gilroy Inc. has been hired to collect the samples and administer the questionnaires for Health and Welfare Canada.

Below you will find information on the purpose of the study, how it will be done, and your part in the study. Also described are the risks, inconveniences, discomforts and other important information that you will need to make a decision about participating in our project. Please feel free to discuss any questions you have with the project staff. We want you to clearly understand the study and your potential role in it. You can talk to the project staff by calling Goss Gilroy Inc. at 1-800-463-8236.

Purpose

The purpose of this study is to look at fish consumption patterns and to compare levels of environmental pollutants in people who eat large amounts of sport fish from the Great Lakes or St. Lawrence River with levels in people who do not eat any sport fish from the Great Lakes or St. Lawrence River.

Participation

Your participation in this study is completely voluntary. You may withdraw at any time. Throughout the study please feel free to ask any questions regarding the project.

Description and Explanation of the Study Procedure(s)

Each participant is being asked to provide a urine sample, a hair sample, and a blood sample, and to complete a questionnaire. The urine, hair and blood samples will be analyzed for (1) selected environmental pollutants such as PCBs, pesticides and mercury; (2) blood lipid (fat) levels and clinical chemistry profile and (3) other measurements (called biomarkers) such as enzyme levels.

Members of the study team will come to your home or another suitable location of your choice at a time convenient for you to collect the samples and to administer the questionnaire.

You will be asked to not eat any fish for three days before the visit. Prior to the visit, you will be given a plastic, leak-proof container for collection of the urine sample and a brown paper bag to keep the light away from the sample. The morning of your interview, you will be asked to collect your first morning urine. The closed container is to be placed in the brown paper bag and stored in a refrigerator. The specimen will be picked up by a member of the study team at the time of your interview. This urine sample will be analyzed only for biomarkers.

A hair sample will be collected from the back of your head. It will be taken at the scalp and it will be about the size of a pencil in diameter. This hair sample will be analyzed for mercury levels.

A blood sample will be collected from your arm for laboratory analysis of certain environmental pollutants, a lipid and clinical chemistry profile, liver enzymes and other biomarkers.

The interview will consist of answering a detailed dietary questionnaire. The questions will focus on your freshwater fish eating habits during the last year. There will also be questions about your general health. We would like you to answer all questions as thoroughly and accurately as possible.

Possible Risks and Discomforts

The only risk or discomfort involved will be associated with taking the blood sample. There may be some slight discomfort when the needle is inserted in the arm to collect blood. There may also be mild swelling or bruising at the site due to bleeding under the skin. These concerns are normally associated with any blood test. All procedures will be carried out under sterile conditions by a person trained in taking blood samples.

Sample Results

The results of the tests will not be available until many months after the collection because of the small number of samples that can be analyzed by the laboratory each day. When the results are available, a summary of your results will be sent to you in the mail. So that we can contact you if you move, you will be asked to provide us with the name and address of someone who will know your new address. You can choose to have the results sent to your family doctor as well. Dr. Cole, the study's medical advisor, will review all results of concern and will be available to discuss them with your doctor.

Confidentiality

To maintain confidentiality, participants will be identified by a number. The information on individual results and names will only be available to Health and Welfare Canada (including the medical advisor to the project), Goss Gilroy Inc. (during the study period only) and your family doctor, if you have requested so.

Reports will be prepared which summarize the overall results and conclusions of the study. These will be available to the public and the scientific community, however no individuals will be identified in these reports.

Appendix 3
Stage 2 questionnaire
(interviewer administered)

Stage 2 Questionnaire
Great Lakes Basin Anglers Survey

Sections

A. Freshwater and saltwater fish consumption, p.2
B. Wildfowl and game consumption, p. 10
C. Smoking/alcohol consumption, p. 12
D. General information, p. 13
E. Health history, p. 17
F. Future Contact, p, 19

SECTION A IDENTIFICATION

Identification Number : ______________

Date of interview: _________________

Sex: Male ○ Female ○

Date of Birth: Day ____ Month ____ Year _____

For Interviewer Only

Interviewer Code __________

Questionnaire	Complete	○	Incomplete	○
	Reliable	○	Unreliable	○

Total Number Pages in Questionnaire after Interview _______

Submitted Questionnaire Checked by: _________________ Supervisor

_________________ Coordinator

SECTION A CONSUMPTION OF FRESH-WATER AND SALTWATER FISH

A1. **Have you ever eaten any fresh-water fish?** *(Check one only)*
1 ○ Yes → *Go to next question*
2 ○ No → *Go to ~~Section B "Wildfowl and Game"~~* 9 15

A2. For approximately how many years have you been eating fresh-water fish?

__ __ years

A3. During which years did you eat fresh-water fish? *(Mark all years or groups of years that apply)* Were there any gaps in this time period where you did not eat any fresh-water fish?

1 ○ before 1930 2 ○ between 1930-39 3 ○ between 1940-49

'50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69
'70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92

A4. During the past 12 months have you eaten any fresh-water fish?
1 ○ Yes → *Go to question A7.*
2 ○ No

A5. Why have you stopped eating fresh-water fish?
(Do not read list to interviewee. Check all that apply)

1 ○ Have not eaten any during past 12 months but will likely eat it in future
2 ○ Now do only catch and release fishing (I don't eat the fish I catch)
3 ○ No longer have time to go fishing
4 ○ No longer have equipment or money needed
5 ○ Lost interest in fishing
6 ○ Not enough fish available to make fishing satisfying
7 ○ No longer receive fish from others
8 ○ No longer buy fish (from restaurants, stores, other fishermen because of cost, availability, overall quality etc.)
9 ○ Concerned about contaminants in fish → What are those concerns? ____________________
10 ○ No specific reason
11 ○ Other (please specify) ____________________

A6. The fresh-water fish that you used to eat, where was most of it caught?
Use location prompts and/or maps to identify up to 5 locations which account for most fish eaten in the past.
1. ____________________
2. ____________________
3. ____________________
4. ____________________
5. ____________________

→ *GO TO SECTION ~~B - "WILDFOWL AND GAME"~~* 9 15

Fresh Water Fish Eaten during the past 12 months

A7. *(Give Reference Card)* From which of the sources listed on the card did you get most of the fresh water fish that you ate during the past 12 months? What was the next most frequent source *(continue for all sources)*?

Rank as 1,2,3,4,5,9
1 - most often
5 - least often
9 - never

1 __ Caught by you?
2 __ Caught by other members of your household?
3 __ Given to you by others?
4 __ Purchased at a store or from an individual?
5 __ Eaten outside your home (restaurant, other's homes etc.)?

A8. Where were the fresh-water fish that you ate during the past 12 months, caught? We would like you to be as specific about the locations as possible. If caught in one of the Great Lakes or any of the main waters or channels connected to them, please be specific about what section (bay, harbour, nearest community). For the lakes, we would also like to know if the fish were caught near the shore or further out into the lake.

For inland rivers and lakes in Ontario give the name of the waterway and the section (name of nearest community). All inland rivers and lakes outside Ontario need only be reported as province or state ... no other detail of location is needed. I have a map to help identify each location, if you need it.

For fish that was given to you or that you bought or ate outside your home, please give any information that you know about where it was caught.

Also inquire about fish 1) purchased at stores, 2) restaurants and 3) gifts from friends or eaten in friends' homes. For each of these categories record, if known, the locations where the fish were caught (e.g. store bought - L. St. Francis or "restaurant - L. Huron"). If no information about the source is available, the source will be "unknown" (e.g. store bought - unknown source" or "restaurant - unknown source". For each seperate category record "unknown").

Record each location on a separate line:

1. ______________________
2. ______________________
3. ______________________
4. ______________________
5. ______________________
6. ______________________
7. ______________________
8. ______________________
9. ______________________
10. ______________________
11. ______________________
12. ______________________
13. ______________________
14. ______________________

A9. **Frequencies of Consumption of Various Species**

"In the next section we want to know what kind of fish you ate from all the locations you mentioned. Then for each kind of fish we want to know how often you ate it and during what season. Lets begin with *(name location)*. What fish did you eat from there? Please give your answer according to how often you ate it during each of the past 4 seasons. Report this as the number of times per week, per month, or for the whole season, whichever is easiest for you.

(Coding: number/d(day) or no./w(week) or no./m(month) or no./s(season)or no./y(year))

Frequency of Consumption of Fish Species from ____________________

Seasonal Consumption of Fish By Species

Species	Consumed	Fall, 1991 Oct.-Dec. (13 weeks)	Winter Jan.-March (13 weeks)	Spring Apr.-June (13 weeks)	Summer July-Sept. (13 weeks)	Last 12 months
Largemouth Bass	○	__/__	__/__	__/__	__/__	__/__
Smallmouth Bass	○	__/__	__/__	__/__	__/__	__/__
White Bass	○	__/__	__/__	__/__	__/__	__/__
Bass, unknown species	○	__/__	__/__	__/__	__/__	__/__
Yellow (Lancaster) Perch	○	__/__	__/__	__/__	__/__	__/__
White Perch	○	__/__	__/__	__/__	__/__	__/__
Pike, Northern	○	__/__	__/__	__/__	__/__	__/__
Walleye (Yellow Pickerel)	○	__/__	__/__	__/__	__/__	__/__
Sauger	○	__/__	__/__	__/__	__/__	__/__
Lake Trout	○	__/__	__/__	__/__	__/__	__/__
Brown Trout	○	__/__	__/__	__/__	__/__	__/__
Brook (Speckled) Trout	○	__/__	__/__	__/__	__/__	__/__
Rainbow Trout	○	__/__	__/__	__/__	__/__	__/__
Splake (cross of lake & brook trout)	○	__/__	__/__	__/__	__/__	__/__
Trout, unknown species	○	__/__	__/__	__/__	__/__	__/__
Coho Salmon	○	__/__	__/__	__/__	__/__	__/__
Chinook salmon	○	__/__	__/__	__/__	__/__	__/__
Atlantic salmon	○	__/__	__/__	__/__	__/__	__/__
Pink salmon	○	__/__	__/__	__/__	__/__	__/__
Salmon, unknown species	○	__/__	__/__	__/__	__/__	__/__
Whitefish	○	__/__	__/__	__/__	__/__	__/__
Cisco (Lake Herring)	○	__/__	__/__	__/__	__/__	__/__
Brown Bullhead	○	__/__	__/__	__/__	__/__	__/__
Channel Catfish	○	__/__	__/__	__/__	__/__	__/__
Sucker (White/Redhorse)	○	__/__	__/__	__/__	__/__	__/__
Freshwater Drum	○	__/__	__/__	__/__	__/__	__/__
Bowfin	○	__/__	__/__	__/__	__/__	__/__
Burbot, Ling	○	__/__	__/__	__/__	__/__	__/__
Pumpkinseed (Sunfish)	○	__/__	__/__	__/__	__/__	__/__
Bluegill	○	__/__	__/__	__/__	__/__	__/__
Rock Bass (Redeye)	○	__/__	__/__	__/__	__/__	__/__
Black Crappie	○	__/__	__/__	__/__	__/__	__/__
Panfish, unknown species	○	__/__	__/__	__/__	__/__	__/__
Carp	○	__/__	__/__	__/__	__/__	__/__
American Eel	○	__/__	__/__	__/__	__/__	__/__
Rainbow Smelt	○	__/__	__/__	__/__	__/__	__/__
Other ______________	○	__/__	__/__	__/__	__/__	__/__
Other ______________	○	__/__	__/__	__/__	__/__	__/__
Other ______________	○	__/__	__/__	__/__	__/__	__/__
Other ______________	○	__/__	__/__	__/__	__/__	__/__
Fish Roe *(species)* __________	○	__/__	__/__	__/__	__/__	__/__
__________	○	__/__	__/__	__/__	__/__	__/__
Fish Liver *(species)* __________	○	__/__	__/__	__/__	__/__	__/__
__________	○	__/__	__/__	__/__	__/__	__/__
Other fish organs *(species)*						
______________	○	__/__	__/__	__/__	__/__	__/__
______________	○	__/__	__/__	__/__	__/__	__/__

Repeat this layout for each location. Extra copies to be added as needed.

Comments:

Frequency of Consumption of Fish Species from ____________________

Seasonal Consumption of Fish By Species

Species	Consumed	Fall, 1991 Oct.-Dec. (13 weeks)	Winter Jan.-March (13 weeks)	Spring Apr.-June (13 weeks)	Summer July-Sept. (13 weeks)	Last 12 months
Largemouth Bass	○	__/__	__/__	__/__	__/__	__/__
Smallmouth Bass	○	__/__	__/__	__/__	__/__	__/__
White Bass	○	__/__	__/__	__/__	__/__	__/__
Bass, unknown species	○	__/__	__/__	__/__	__/__	__/__
Yellow (Lancaster) Perch	○	__/__	__/__	__/__	__/__	__/__
White Perch	○	__/__	__/__	__/__	__/__	__/__
Pike, Northern	○	__/__	__/__	__/__	__/__	__/__
Walleye (Yellow Pickerel)	○	__/__	__/__	__/__	__/__	__/__
Sauger	○	__/__	__/__	__/__	__/__	__/__
Lake Trout	○	__/__	__/__	__/__	__/__	__/__
Brown Trout	○	__/__	__/__	__/__	__/__	__/__
Brook (Speckled) Trout	○	__/__	__/__	__/__	__/__	__/__
Rainbow Trout	○	__/__	__/__	__/__	__/__	__/__
Splake (cross of lake & brook trout)	○	__/__	__/__	__/__	__/__	__/__
Trout, unknown species	○	__/__	__/__	__/__	__/__	__/__
Coho Salmon	○	__/__	__/__	__/__	__/__	__/__
Chinook salmon	○	__/__	__/__	__/__	__/__	__/__
Atlantic salmon	○	__/__	__/__	__/__	__/__	__/__
Pink salmon	○	__/__	__/__	__/__	__/__	__/__
Salmon, unknown species	○	__/__	__/__	__/__	__/__	__/__
Whitefish	○	__/__	__/__	__/__	__/__	__/__
Cisco (Lake Herring)	○	__/__	__/__	__/__	__/__	__/__
Brown Bullhead	○	__/__	__/__	__/__	__/__	__/__
Channel Catfish	○	__/__	__/__	__/__	__/__	__/__
Sucker (White/Redhorse)	○	__/__	__/__	__/__	__/__	__/__
Freshwater Drum	○	__/__	__/__	__/__	__/__	__/__
Bowfin	○	__/__	__/__	__/__	__/__	__/__
Burbot, Ling	○	__/__	__/__	__/__	__/__	__/__
Pumpkinseed (Sunfish)	○	__/__	__/__	__/__	__/__	__/__
Bluegill	○	__/__	__/__	__/__	__/__	__/__
Rock Bass (Redeye)	○	__/__	__/__	__/__	__/__	__/__
Black Crappie	○	__/__	__/__	__/__	__/__	__/__
Panfish, unknown species	○	__/__	__/__	__/__	__/__	__/__
Carp	○	__/__	__/__	__/__	__/__	__/__
American Eel	○	__/__	__/__	__/__	__/__	__/__
Rainbow Smelt	○	__/__	__/__	__/__	__/__	__/__
Other ________________	○	__/__	__/__	__/__	__/__	__/__
Other ________________	○	__/__	__/__	__/__	__/__	__/__
Other ________________	○	__/__	__/__	__/__	__/__	__/__
Other ________________	○	__/__	__/__	__/__	__/__	__/__
Fish Roe *(species)* __________	○	__/__	__/__	__/__	__/__	__/__
__________	○	__/__	__/__	__/__	__/__	__/__
Fish Liver *(species)* __________	○	__/__	__/__	__/__	__/__	__/__
__________	○	__/__	__/__	__/__	__/__	__/__
Other fish organs *(species)*						
______________	○	__/__	__/__	__/__	__/__	__/__
______________	○	__/__	__/__	__/__	__/__	__/__

Repeat this layout for each location. Extra copies to be added as needed.

Comments:

Frequency of Consumption of Fish Species from ____________________

Seasonal Consumption of Fish By Species

Species	Consumed	Fall, 1991 Oct.-Dec. (13 weeks)	Winter Jan.-March (13 weeks)	Spring Apr.-June (13 weeks)	Summer July-Sept (13 weeks)	Last 12 months
Largemouth Bass	○	__/__	__/__	__/__	__/__	__/__
Smallmouth Bass	○	__/__	__/__	__/__	__/__	__/__
White Bass	○	__/__	__/__	__/__	__/__	__/__
Bass, unknown species	○	__/__	__/__	__/__	__/__	__/__
Yellow (Lancaster) Perch	○	__/__	__/__	__/__	__/__	__/__
White Perch	○	__/__	__/__	__/__	__/__	__/__
Pike, Northern	○	__/__	__/__	__/__	__/__	__/__
Walleye (Yellow Pickerel)	○	__/__	__/__	__/__	__/__	__/__
Sauger	○	__/__	__/__	__/__	__/__	__/__
Lake Trout	○	__/__	__/__	__/__	__/__	__/__
Brown Trout	○	__/__	__/__	__/__	__/__	__/__
Brook (Speckled) Trout	○	__/__	__/__	__/__	__/__	__/__
Rainbow Trout	○	__/__	__/__	__/__	__/__	__/__
Splake (cross of lake & brook trout)	○	__/__	__/__	__/__	__/__	__/__
Trout, unknown species	○	__/__	__/__	__/__	__/__	__/__
Coho Salmon	○	__/__	__/__	__/__	__/__	__/__
Chinook salmon	○	__/__	__/__	__/__	__/__	__/__
Atlantic salmon	○	__/__	__/__	__/__	__/__	__/__
Pink salmon	○	__/__	__/__	__/__	__/__	__/__
Salmon, unknown species	○	__/__	__/__	__/__	__/__	__/__
Whitefish	○	__/__	__/__	__/__	__/__	__/__
Cisco (Lake Herring)	○	__/__	__/__	__/__	__/__	__/__
Brown Bullhead	○	__/__	__/__	__/__	__/__	__/__
Channel Catfish	○	__/__	__/__	__/__	__/__	__/__
Sucker (White/Redhorse)	○	__/__	__/__	__/__	__/__	__/__
Freshwater Drum	○	__/__	__/__	__/__	__/__	__/__
Bowfin	○	__/__	__/__	__/__	__/__	__/__
Burbot, Ling	○	__/__	__/__	__/__	__/__	__/__
Pumpkinseed (Sunfish)	○	__/__	__/__	__/__	__/__	__/__
Bluegill	○	__/__	__/__	__/__	__/__	__/__
Rock Bass (Redeye)	○	__/__	__/__	__/__	__/__	__/__
Black Crappie	○	__/__	__/__	__/__	__/__	__/__
Panfish, unknown species	○	__/__	__/__	__/__	__/__	__/__
Carp	○	__/__	__/__	__/__	__/__	__/__
American Eel	○	__/__	__/__	__/__	__/__	__/__
Rainbow Smelt	○	__/__	__/__	__/__	__/__	__/__
Other ________________	○	__/__	__/__	__/__	__/__	__/__
Other ________________	○	__/__	__/__	__/__	__/__	__/__
Other ________________	○	__/__	__/__	__/__	__/__	__/__
Other ________________	○	__/__	__/__	__/__	__/__	__/__
Fish Roe *(species)* __________	○	__/__	__/__	__/__	__/__	__/__
____________	○	__/__	__/__	__/__	__/__	__/__
Fish Liver *(species)* __________	○	__/__	__/__	__/__	__/__	__/__
____________	○	__/__	__/__	__/__	__/__	__/__
Other fish organs *(species)*						
________________	○	__/__	__/__	__/__	__/__	__/__
________________	○	__/__	__/__	__/__	__/__	__/__

Repeat this layout for each location. Extra copies to be added as needed.

Comments:

10. Preparation, Cooking and Serving Size

Species	Cleaning					Parts Eaten				Usual Serving Size	
	To prepare this species did you?					What parts did you eat?				Portion Models	
	cut off and discard the belly meat	cut off and discard the fat from the back	cut off and discard the fat from the sides	remove the skin	puncture the skin	whole fish, less head, fins, tail, guts, and bones	fillets	steaks	skin	Model#	Thickness (steaks, filet pieces only)
Largemouth Bass											
Smallmouth Bass											
White Bass											
Bass, unknown species											
Yellow (Lancaster) Perch											
White Perch											
Pike, Northern											
Walleye (Yelow Pickerel)											
Sauger											
Lake Trout											
Brown Trout											
Brook (Speckled) Trout											
Rainbow Trout											
Splake (cross between lake and brook trout)											
Trout, unknown species											
Coho Salmon											
Chinook Salmon											
Atlantic Salmon											
Pink Salmon											
Salmon, unknown species											
Whitefish											
Cisco (Lake Herring)											
Brown Bullhead											
Channel Catfish											
Sucker (White/Redhorse)											
Freshwater Drum											
Bowfin											
Burbot, Ling											
Pumpkinseed (Sunfish)											
Bluegill											
Rock Bass (Redeye)											
Black Crappie											
Panfish-Unknown species											
Carp											
American Eel											
Rainbow Smelt											
Other ____________											
Other ____________											
Other ____________											
Other ____________											
Fish Roe (species) __________ __________ Fish Liver (species) ___ ______________ Other fish organs: (species) ______________ ______________											

Codes: U(sually)
S(ometimes)

omments:

				Cooking						Preservation		
				To cook this species did you?						To keep it did you?		
Length (for whole fillets only) (no thickness)	# eaten/ meal	weight	From R(aw) C(ooked)	deep-fat fry or pan fry it	re-use any of this frying fat	bake, broil, grill, or BBQ it	poach, boil, microwave, or steam it	use it in soups, chowders, stews	use it in sandwiches, casseroles, fish cakes	can, pickle or salt it	smoke it	freeze it

Species	✓
Largemouth Bass	
Smallmouth Bass	
White Bass	
Bass, unknown species	
Yellow (Lancaster) Perch	
White Perch	
Pike, Northern	
Walleye (Pickerel)	
Sauger	
Lake Trout	
Brown Trout	
Brook (Speckled) Trout	
Rainbow Trout	
Splake	
Trout, unknown species	
Coho Salmon	
Chinook Salmon	
Atlantic Salmon	
Pink Salmon	
Salmon, unknown species	
Whitefish	
Cisco (Lake Herring)	
Brown Bullhead	
Channel Catfish	
Sucker (White/Redhorse)	
Freshwater Drum	
Bowfin	
Burbot, Ling	
Pumpkinseed (Sunfish)	
Bluegill	
Rock Bass (Redeye)	
Black Crappie	
Panfish, unknown species	
Carp	
American Eel	
Rainbow Smelt	
Other__________	
Other__________	
Other __________	
Other__________	
Fish Roe __________ __________ Fish Liver __________ __________ Other fish organs __________ __________	

A11. **Describe any methods you use for selecting fish for eating.** ***(prompt only if necessary: For example personal guidelines you use regarding size, species, location, etc.)***

____

____

____

____

A12. **Is there anything else you do to limit your exposure to environmental contaminants from fish? If yes, please describe them.** ***(If new information arises that impacts on A10, then go back)***

____

____

____

____

A13. **(a) Has your consumption of freshwater fish changed in the last 10 years?** ***(eg. location, species, how often)***

^{1} ○ Yes
^{2} ○ No → Go to Q. A14

(b) Compared to 10 years ago, is the amount of fresh water fish you eat now greater, lesser, or about the same?

^{1} ○ Greater amount now
^{2} ○ Lesser amount now
^{3} ○ About the same

When this change occur?

^{1} ○ Gradually since 19 _ _
^{2} ○ Abrupt change in 19 _ _
^{3} ○ Can't say exactly

(c) Are you eating different species now than you did 10 years ago?

^{1} ○ Yes
^{2} ○ No

If YES, name species you used to eat:

a. ______________________
b. ______________________
c. ______________________
d. ______________________

When this change occur?

^{1} ○ Gradually since 19 _ _
^{2} ○ Abrupt change in 19 _ _
^{3} ○ Can't say exactly

(d) Are there locations or sources of sport fish that you no longer use?

^{1} ○ Yes
^{2} ○ No

If YES, what are they?

a. ______________________________
b. ______________________________
c. ______________________________
d. ______________________________

When this change occur?

^{1} ○ Gradually since 19 _ _
^{2} ○ Abrupt change in 19 _ _
^{3} ○ Can't say exactly

A14. The Ontario Ministries of Environment and Natural Resources publish an annual "Guide to Eating Ontario Sport Fish". *(show a copy of the guide)*

a) Were you aware that such a Guide exists?
^{1} ○ Yes
^{2} ○ No → *Go to A15*

b) Do you have a copy of this Guide?
^{1} ○ Yes
^{2} ○ No → *Go to A15*

c) What is the year of publication of your most recent copy?
19 _ _.

d) How often do you follow the consumption advice in the guide?
^{1} ○ never → *Go to A15*
^{2} ○ sometimes
^{3} ○ usually
^{4} ○ always

e) During which year did you start following the recommendations in the Guide?
19 _ _

A15. How often in the past 12 months have you eaten salt-water fish, that is, fish from the oceans or open seas such as haddock, cod, tuna, sole etc. Do not include here any of the fish from the Great Lakes, St. Lawrence or any other inland lakes or rivers that we covered earlier. We are interested in all ocean fish: that bought in stores, in restaurants, from take-outs, eaten at home etc.

Type of Salt-Water Fish Eaten	Number of Times eaten in the last 12 months			Comments
	Per Week	Per Month	Per Year	
Fillets or Steaks - fresh, frozen, salted (cod, halibut, sole, haddock, ocean salmon etc.)				
Frozen Fish Sticks or Fish Dinners				
Shellfish (lobster, crab, shrimp, scallops, oysters, clams, mussels)				
Canned Fish (tuna, salmon, sardines)				
Whole Fish - fresh or frozen (herring, mackerel, sea smelts)				
Caviar/fish roe				
Other: (Please specify) a. ______________				
b. ______________				
c. ______________				

Comments: ____

____

A16. In the last week (7 days) what fish meals have you eaten? *(Include both freshwater and saltwater fish meals)*

○ No fish meals eaten in last 7 days

Date	Species/Type	Where was it caught/purchased	Number of Meals

Comments: ____

____

A17. When did you eat your last meal?
_______ hours ago.

SECTION B WILDFOWL AND GAME CONSUMPTION

B1. Have you ever eaten any wildfowl or game?
^{1} ○ Yes
^{2} ○ No → *Go to Section C "Smoking and alcohol consumption".*

B2. Approximately how many years have you been eating wildfowl or game?

_ _ years

B3. During which years did you eat wildfowl or game?
(Circle all years or groups of years that apply)

^{1}○ before 1930 ^{2}○ between 1930-39 ^{3}○ between 1940-49

^{4}50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69
70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92

B4. During the past 12 months have you eaten any wildfowl or game?
^{1} ○ Yes → *Go to Q. B6.*
^{2} ○ No

B5. Why have you stopped eating wildfowl and game?
(Do not read to interviewee. Check all that apply)

^{1} ○ Have not eaten any during past 12 months but will likely eat it in future
^{2} ○ No longer have time to hunt
^{3} ○ No longer have equipment or money needed
^{4} ○ Lost interest in hunting wildfowl and game
^{5} ○ Not enough birds or game to make hunting satisfying
^{6} ○ No longer receive wildfowl or game from others
^{7} ○ No longer buy wildfowl or game (restaurants, stores, other hunters — because of cost, availability, overall quality etc)
^{8} ○ Concerned about contaminants in wildfowl and game
^{9} ○ No specific reason
^{10} ○ Other (please specify) ______________________________

→ *Go to C "Smoking and Alcohol Consumption"*

B6. During the past 12 months at approximately how many different meals in total, did you eat the following types of wildfowl or game? *(Give card).* For ducks, geese and game birds we would like to know all the different species you ate. And, for ducks, geese, bullfrogs and turtles, we would like to know the location where you got most of them.

Wildfowl or Game	Specify species when known	Number of meals in last 12 months	Most common location
Wild, fish-eating ducks (specify species)	a.		
	b.		
	c.		
Other wild ducks or geese (specify species)	a.		
	b.		
	c.		
Wild duck or geese eggs (specify species)	a.		
	b.		
	c.		
Other wild birds (specify species)	a.		
	b.		
	c.		
Wild bird eggs	a.		
	b.		
	c.		
Bullfrogs	XXXXXXXXXXXXXXX		
Turtles	XXXXXXXXXXXXXXX		
Turtle Eggs	XXXXXXXXXXXXXXX		
Rabbits or Hare	XXXXXXXXXXXXXXX		
Moose	XXXXXXXXXXXXXXX		
Deer	XXXXXXXXXXXXXXX		
Other Game (specify species)	a.		
	b.		
	c.		
Organ Meats from Wild Fowl or Game (liver/ kidney/ gall)	a.		
	b.		
	c.		

B7. In the last week (7 days) what wildfowl or game have you eaten?

○ No wildfowl/game meals eaten in last 7 days

Date	Species/Type	Where was it caught/purchased	Number of Meals

SECTION C SMOKING/ALCOHOL CONSUMPTION

C1. Are you currently smoking 1 or more cigarettes, cigars or pipes per day?

[1] ○ Yes → a) How many cigarettes, cigars or pipes per day are you smoking?
________ cigarettes per day
________ cigars per day
________ pipes per day

b) How many years have you been smoking?
________ years → *Go to Q. C2.*

[2] ○ No → a) Did you ever smoke 1 or more cigarettes, cigars or pipes per day for at least 1 year?
[1] ○ Yes
[2] ○ No → *Go to Q. C2.*
a) How many cigarettes, cigars or pipes did you smoke per day?
____________ cigarettes per day
____________ cigars per day
____________ pipes per day

b) How many years did you smoke?
________ years

c) When did you quit?
19 __ __

C2. Do you currently chew tobacco or snuff?
[1] ○ Yes →
a) How many plugs of tobacco do you chew per day?
______ plugs per day

b) How many years have you been chewing tobacco?
______ years → *Go to Q. C3.*

[2] ○ No
a) Were you ever a regular chewer of tobacco (1 or more plugs per day for at least 1 year)?
[1] ○ Yes
[2] ○ No → *Go to Q. C3.*
a) How many plugs did you chew per day?
______ plugs per day

b) How many years did you chew tobacco?
______ years

c) When did you quit? 19 __ __

C3. How often in the last 12 months have you had the following alcoholic beverages? For each give the average number of drinks from April to September and during October to March according to how often you consumed these beverages during each period. Report this as the number of times per week, per month, or for the whole period, whichever is easiest for you. *(Coding: number/d(day) or no./w(week) or no./m(month) or no./s(season)or no./[illegible])*

Beverages	Reference Size	Average Number of Drinks	
		April - Sept.	Oct. - March
Beer - regular or lite*	1 can/1 bottle	B1 2	B2
Wine - table or sweet	5 oz		[illegible]
Wine or Liquor Coolers	1 bottle		[illegible]
Hard Liquor and Liqueurs	1 oz		

* exclude 0.5% or non-alcoholic beer

C4. When did you last drink an alcoholic beverage?

_____ hours ago
_____ days ago
_____ weeks ago

SECTION D GENERAL INFORMATION

D1. What is your current employment status? *(Check all that apply.)*

[1] ○ Employed part-time
[2] ○ Employed full-time
[3] ○ Currently Unemployed
[4] ○ Homemaker
[5] ○ On sick leave or maternity leave
[6] ○ Not working due to permanent disability
[7] ○ Retired
[8] ○ Student

D2. We would like some information on your present or most recent job. If you are (or were last year) working at more than one job, please describe each job starting with the one where you work (or worked) the most hours. *(Use a separate copy of this question for each different job).*

CURRENT (OR MOST RECENT) JOB

a) What kind of business, industry or service do you (or did you) work for? *Ask for a full description. For example, dairy farm, high school, insurance agency, automobile manufacturing plant.*

______________________________ ☐

b) What kind of work do you (or did you) do in your job? *For example, dairy farmer, english teacher, accountant, assembly line worker.*

______________________________ ☐

c) What are (or were) your usual duties or activities in this job? *For example, milking cows, teaching english, welding frames.*

______________________________ ☐

d) Date started ____ ____
Month Year
Date stopped (if applicable) ____ ____
Month Year

OTHER CURRENT (OR MOST RECENT) JOB

e) What kind of business, industry or service do you (or did you) work for? *Ask for a full description. For example, dairy farm, high school, insurance agency, automobile manufacturing plant.*

______________________________ ☐

f) What kind of work do you (or did you) do in your job? *For example, dairy farmer, english teacher, accountant, assembly line worker.*

______________________________ ☐

g) What are (or were) your usual duties or activities in this job? *For example, milking cows, teaching english, welding frames.*

______________________________ ☐

h) Date started ____ ____
Month Year
Date stopped (if applicable) ____ ____
Month Year

LONGEST HELD JOB

D3. What job did you work at the longest during your adult life?

○ same as current job above
○ same as second job above OR

a) What kind of business, industry or service do you (or did you) work for? *Ask for a full description. For example, dairy farm, high school, insurance agency, automobile manufacturing plant.*

______________________________ ☐

b) What kind of work do you (or did you) do in your job? *For example, dairy farmer, english teacher, accountant, assembly line worker.*

______________________________ ☐

c) What are (or were) your usual duties or activities in this job? *For example, milking cows, teaching english, welding frames.*

______________________________ ☐

d) Date started ___ ___
Month Year
Date stopped (if applicable) ___ ___
Month Year

D4. In your job, when and how often have you ever come into contact with any of the following materials? Express as number of times per day (D) or week (W) per month (M) or per year (Y).

		Never	Give dates Month/Year TO Month/Year	No. of times	Use personal protective equipment?	
					Yes	No
a)	Metal dust or fumes (e.g. soldering, welding)	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
b)	Fumes from solvents, paints, glues, or varnishes	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
c)	Resins or isocyanates	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
d)	Pesticides (bug or weed killers)	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
e)	Coal tar or pitch	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
f)	Fumes from gasoline, diesel, kerosene or fuel oils	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
g)	Mercury vapours	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
h)	Electrical transformers	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
i)	Radiation	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
j)	Polychlorinated Biphenyls (PCB's)	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
k)	Ethylene oxide	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
l)	Explosives	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
m)	Plastics	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
n)	Photographic developing chemicals	○	______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
			______ to ______	___ per ___	○	○
o)	Other ______________		______ to ______	___ per ___	○	○
	______________		______ to ______	___ per ___	○	○
	______________		______ to ______	___ per ___	○	○

D5. In your home (eg hobbies, crafts, work around home), when and how often have you ever come into contact with any of the following materials? Express as number of times per day (D) or week (W) per month (M) or per year (Y).

					Use personal protective equipment?	
		Never	Give dates Month/Year TO Month/Year	No. of times	Yes	No
a)	Metal dust or fumes (e.g.	○	_______ to _______	___ per ___	○	○
	(such as from soldering, welding,		_______ to _______	___ per ___	○	○
	casting of lead shot or sinkers)		_______ to _______	___ per ___	○	○
b)	Fumes from solvents, paints, glues,	○	_______ to _______	___ per ___	○	○
	or varnishes		_______ to _______	___ per ___	○	○
			_______ to _______	___ per ___	○	○
c)	Resins or isocyanates	○	_______ to _______	___ per ___	○	○
			_______ to _______	___ per ___	○	○
			_______ to _______	___ per ___	○	○
d)	Pesticides (bug or weed killers)	○	_______ to _______	___ per ___	○	○
	(own lawn only)		_______ to _______	___ per ___	○	○
			_______ to _______	___ per ___	○	○
e)	Fumes from gasoline, diesel,	○	_______ to _______	___ per ___	○	○
	kerosene or fuel oils		_______ to _______	___ per ___	○	○
			_______ to _______	___ per ___	○	○
f)	Photographic developing chemicals	○	_______ to _______	___ per ___	○	○
			_______ to _______	___ per ___	○	○
			_______ to _______	___ per ___	○	○
g)	Other ____________________	○	_______ to _______	___ per ___	○	○

D6. Are any of the of following located within 100 metres from your home?

		No	Yes
a)	Golf Course	○	○
b)	Dry cleaning plant	○	○
c)	Auto repair garage	○	○
d)	Parking garage	○	○
e)	Highway bridge	○	○
f)	Chemical factory	○	○
g)	Toxic waste or garbage dump	○	○
i)	Other (specify) _______________		

D7. What is the highest grade (or year) of schooling that you have completed? *(check one)*

^{1} ○ Any of Grades 1 to 8 (or equivalent)
^{2} ○ Any of Grades 9 to 11 (or equivalent)
^{3} ○ High School Graduate (or equivalent)
^{4} ○ Started college, trade school or university but did not complete
^{5} ○ Community College or Trade School Diploma or Certificate
^{6} ○ University Degree
^{7} ○ No formal education

D8. We'd like to ask this NEXT question simply to help us categorize your answers with others like you. We don't need to know actual amounts, only the group letter from this card. Which of these categories represents the total income earned by you, and all members of your household combined, in 1991, before taxes. *(Present the Income Card).*

A ^{1} ○ less than \$15,000
B ^{2} ○ \$15,000 - \$29,999
C ^{3} ○ \$30,000 - \$44,999
D ^{4} ○ \$45,000 - \$59,999
E ^{5} ○ \$60,000 or more
^{6} ○ Don't know
^{7} ○ No response

D9. How many adults (including yourself) and how many children are supported by this income? *(Enter number.)*

____ adults

____ children (less than 18 years)

SECTION E HEALTH HISTORY

E1. We would like to know if you have ever been told by a doctor that you had (or have) any of the following health problems.

(To Interviewer: Read each condition in turn and complete the information required.)

Disease/Condition	Ever Had (YES or NO)	If YES: Year of Diagnosis
a) Diabetes	1 ○ Yes → 2 ○ No	19 __ __
b) Celiac Disease	1 ○ Yes → 2 ○ No	19 __ __
c) Hepatitis	1 ○ Yes → 2 ○ No	19 __ __
d) High cholesterol	1 ○ Yes → 2 ○ No	19 __ __
e) Irritable bowel syndrome	1 ○ Yes → 2 ○ No	19 __ __
f) Liver disease	1 ○ Yes → 2 ○ No	19 __ __
g) Thyroid disease or Goiter	1 ○ Yes → 2 ○ No	19 __ __
h) Colitis	1 ○ Yes → 2 ○ No	19 __ __

Comments:

E2. a) Are you currently using prescription drugs (*women only* - including contraceptives)?
1 ○ Yes
2 ○ No → *Go to Q. E3.*

b) If YES please list the prescription drugs that you are currently taking, the dosage of the drug, and the length of time you have been taking the medicine:

Name of Drug	Dosage			Length of Time Using Drug	
	strength (mg)	# tablets / spoons	# times per day		
1.					
2.					
3.					
4.					
5.					
6.					
7.					

E3. Are you currently on any specialized diet prescribed by your physician?
[1] ○ Yes → Which type? ________________
[2] ○ No

E4. a) Have you ever had chemotherapy for cancer or a tumour?
[1] ○ Yes
[2] ○ No → *Go to Q. E5.*

b) Has this chemotherapy treatment been completed?
[1] ○ Yes
[2] ○ No

c) When was the last treatment?

____ Year → if 1992 then ____ Month

E5. a) Have you ever had radiation therapy for cancer or a tumour?
[1] ○ Yes
[2] ○ No → *Go to Q. E6.*

b) Has this radiation therapy been completed?
[1] ○ Yes
[2] ○ No

c) When was the last treatment?
____ Year → if 1992 then _____ Month

E6. Have you ever had your spleen removed?
[1] ○ Yes
[2] ○ No

E7. a) What is your present height?
___ ft ___ inches OR ____ meters

b) What is your present weight?
____ pounds OR ____ kilograms

c) Have you lost or gained any weight in the last 6 months?
[1] ○ No
[2] ○ Lost weight → _____ lbs / kgs
[3] ○ Gained weight → _____ lbs / kgs

For Men → go to section F Future Contact

E8. FOR WOMEN ONLY (18 and over)

a) Have you had any children of your own?

^{1} ○ Yes
^{2} ○ No → *go to ~~future contact~~* c).

b) We would like to know when each child was born and, if he/she was breast fed, we would like to know how long for.

Child	Date of birth	Breast fed?	Number of months Fully breast fed (no other foods)	Number of Months Partially breast fed (other foods given)
1.		^{1} ○ Yes → ^{2} ○ No	___	___
2.		^{1} ○ Yes → ^{2} ○ No	___	___
3.		^{1} ○ Yes → ^{2} ○ No	___	___
4.		^{1} ○ Yes → ^{2} ○ No	___	___
5.		^{1} ○ Yes → ^{2} ○ No	___	___
6.		^{1} ○ Yes → ^{2} ○ No	___	___
7.		^{1} ○ Yes → ^{2} ○ No	___	___
8.		^{1} ○ Yes → ^{2} ○ No	___	___
9.		^{1} ○ Yes → ^{2} ○ No	___	___

c) Are you pregnant now?
^{1} ○ Yes
^{2} ○ No

SECTION F FUTURE CONTACT

Ask interviewee to please give name, address, telephone number of relative (father, mother, brother) or friend who we could contact if we need to reach them in the future and are unable to do so.

Name: ______________________________
First Name Second Name Family Name

Address: ______________________________
Street / P.O. Box / R.R.# / Apartment #
______________________________ ___ ___
Municipality Province Postal Code

Home telephone number: (___) ___ ___

GOSS GILROY INC.
Management Consultants
Conseillers en gestion

Goss, Gilroy Inc. Suite 900, 150 Metcalfe St. Ottawa, Ontario K2P 1P1

Appendix 4
Methods for calculation of meal size

Methods for calculation of meal size

In question 10, an attempt was made to determine the "usual" meal size for every species which each participant reported eating in the last 12 months. It was felt that for each participant, meal size may differ significantly between species depending on a number of factors such as availability or preference.

Generic fish meal models were prepared from opaque plexiglass (.3 cm thick), as shown in Figure appendix 4-1, to aid in estimating meal size. A thickness indicator (Figure App.4-1) made of 3.3x3.3 cm. squares, was used to estimate the thickness of the fish piece. These models were similar to ones used by the Nutrition Canada Survey for other food items. They were specifically kept generic and simple looking so participants would not be confused if they looked like a different species than the one for which they were reporting. The models covered a range of sizes for 3 basic shapes:

(1) fillets
(2) steaks
(3) fillet pieces (ie. parts of large fillets)

Each participant was asked to specify which was the most usual method used for preparing a particular species for eating.

For steaks or fillet pieces they were then asked to

(1) choose the most appropriate size that they usually ate,
(2) indicate the thickness of the steak or fillet piece, using the thickness indicator
(3) indicate whether the size chosen was for a cooked or raw piece.

These selections were later used to estimate weight (assuming a constant thickness through the piece) by multiplying the surface area of the model x thickness to get a volume. The volume was multiplied by a density estimate for that fish or similar species. How these density estimates were obtained is described in more detail in Figure App.4-2. The estimated weight was then multiplied by the number of pieces which the participant reported eating to obtain a meal size.

Since the shape and thickness profile of fillets differ widely among species, it was felt that it was inappropriate to assume a constant thickness or standard surface areas for the fillets. Consequently, participants were asked to select a fillet model that was of similar length to that which they usually ate (or use the ruler to select a length). Using this length, regression curves were used to predict the weight of the fillet for that species (or similar one). Figure App.4-2 describes how these regression curves were obtained. This weight was multiplied by the number of fillets the participant reported eating at a meal to obtain a meal size.

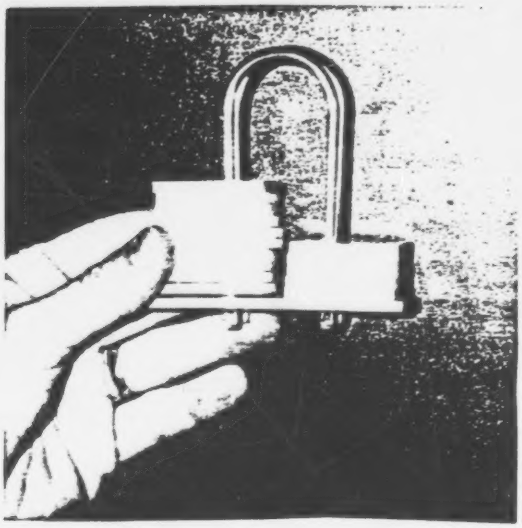
In the case of whole fish, the participant was asked to provide the length of the whole fish (minus head, tail, fins). The whole fish was assumed to be equal to 2 fillets. The length of the dressed whole fish was used to estimate a fillet weight, multiplied by 2 (2 fillets/fish) and times the number of whole fish eaten per meal to obtain total meal size. Sometimes, participants indicated a model size half way between 2 models, which was coded as 'M' and an average was calculated for the 2 selections. Similarly, some participants said they ate, for example, both fillet pieces and steaks. Again, two estimates were calculated and averaged to obtain a meal size.

In total there were 647 meal size data points (participant-species combinations). The following is a breakdown of the type of data used to calculate the mealsize for these records:

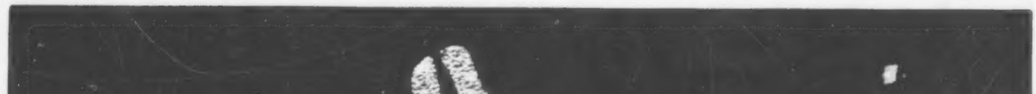
Data used to calculate mealsize	Number of records	Percentage of records
fillet model and regression equation	456	70.5
fillet length and regression equation	33	5.1
'pieces' model, thickness and density estimates	34	5.3
'steak' model, thickness and density estimates	30	4.6
'mixed' model	6	0.9
whole fish length and regression equation	33	5.1
weight of meal given	18	2.8
other methods	13	2.0
meal size missing	24	3.9
Total number of records	647	100.0

The models were used to calculate the meal size in 86.4% of the records with the fillet model being the most common (70.5%).

Figure App.4-1
Fish meal models



Thickness indicator



Clockwise from top: 'steak' models, 'fish pieces' models, 'filet' models & thickness indicator

Figure App. 4-2
Estimation of density, fillet length to weight relations

During the development of the methodology described in this appendix, it became obvious that density estimates and fillet length weight relationships would be needed for raw and cooked fish of each species. Surprisingly little information of this type for freshwater fish could be found in the literature. Consequently, a contract was let to a commercial fisherman, working in Lake Ontario, to obtain fish of a selected group of "representative" species and carry out the appropriate measures of the fish to provide the needed data. Fifteen fish were collected across a range of sizes for the following 11 representative species:

- Smallmouth bass
- Yellow perch
- Northern pike
- Walleye (pickerel)
- Lake trout
- Rainbow trout
- Chinook salmon
- Brown bullhead
- Channel catfish
- Black crappie
- Carp

Extensive measurements were made on the whole fish and the fillets obtained from each fish (see reporting page, Table App. 4-1). Not all measurements were needed for this study but it was felt that since so little data existed, it was a good opportunity to meet other possible needs. The whole fish was weighed before and after cleaning; length, width, thickness at thickest point and volume were measured. The whole fish was then filleted into 2 fillets. Similar measurements, including surface area, were made on each raw fillet. One fillet was then trimmed according to the recommended guidelines provided by the Ontario Ministry of Environment and Energy. All measurements were then repeated for the "trimmed" fillet. Both fillets (one trimmed, one untrimmed) were then cooked and all measurements repeated again.

Density estimates have been obtained using the measurements of surface area and volume from the 2 untrimmed fillets. The fillet length-weight relationships were calculated for the untrimmed fillets, separately for raw and cooked. Table App.4-2 provides a list of which of the 11 'representative' species were used for those species for which no data was collected. The regression equations and density estimates are provided in Table App. 4-3.

Table App. 4-1
DATA COLLECTION SHEET
GREAT LAKES ANGLERS PILOT EXPOSURE ASSESSMENT STUDY

FISH SPECIES		MEASUREMENT	FRESH FISH	DRESSED FISH
LOCATION CAUGHT		Length (cm)		
DATE CAUGHT		Weight (gm)		

MEASUREMENT	LEFT FILLET (UNTRIMMED) (NOT COOKED)	RIGHT FILLET (UNTRIMMED) (NOT COOKED)	LEFT FILLET (TRIMMED) (NOT COOKED)	LEFT FILLET (TRIMMED) (COOKED)	RIGHT FILLET (UNTRIMMED) (COOKED)
Length (cm)					
Width (cm)					
Thickness (cm)					
Weight (gm)					
Surface Area (cm^2)					
Volume (cm^3)					

Table App.4-2
Representative species for fish measurement estimates

"Representative" species	Other species
Small mouth bass	Small mouth, large mouth, white bass, "bass, unknown species", freshwater drum
Brown bullhead	Brown bullhead
Carp	Carp
Channel catfish	Channel catfish
Black crappie	Black crappie, pumpkinseed, rock bass, bluegill, white crappied, "panfish-unknown species"
Lake trout	Lake trout, splake
Yellow perch	Yellow, white perch, "perch, unknown species"
Northern pike	Pike, muskellunge
Rainbow trout	Rainbow, brook, brown trout, "trout, unknown species", cisco, whitefish, sucker
Chinook salmon	Chinook, coho, pink salmon, "salmon, unknown species"
Walleye (pickerel)	Walleye, sauger

Table App.4-3
Regression equations and density estimates

"Representative" species	Regression equations (wt = weight of fillet in grams length = length of fillet in cms.)		Density estimates (gm/cm^3)	
	Raw	Cooked	Raw	Cooked
Smallmouth bass	$\text{wt} = \exp(1.79 + 0.339 * (\ln(\text{length}))^2)$	$\text{wt} = \exp(2.02 + 0.372 * (\ln(\text{length}))^2)$	1.10	1.16
Brown bullhead	$\text{wt} = \exp(0.924 + 0.402 * (\ln(\text{length}))^2)$	$\text{wt} = 0.0377 * (\text{length} ** 3)$	1.12	1.37
Carp	$\text{wt} = \exp(1.31 + 0.401 * (\ln(\text{length}))^2)$	$\text{wt} = \exp(2.54 + 0.353 * (\ln(\text{length}))^2)$	1.05	1.14
Channel catfish	$\text{wt} = \exp(0.998 + 0.390 * (\ln(\text{length}))^2)$	$\text{wt} = \exp(1.29 + 0.44 * (\ln(\text{length})^2)$	1.05	1.23
Black crappie	$\text{wt} = 0.0214 * (\text{length}^3)$	$\text{wt} = 0.0488 * (\text{length}^3)$	1.13	1.21
Lake trout	$\text{wt} = \exp(1.17 + 0.377 * (\ln(\text{length}))^2)$	$\text{wt} = \exp(1.53 + 0.399 * (\ln(\text{length})^2)$	1.07	1.17
Yellow perch	$\text{wt} = \exp(0.507 + (\ln(\text{length}))^2)$	$\text{wt} = 0.0374 * (\text{length}^3)$	1.08	1.26
Northern pike	$\text{wt} = \exp(0.430 + (\ln(\text{length}))^2)$	$\text{wt} = 0.0164 * (\text{length}^3)$	1.09	1.20
Rainbow trout	$\text{wt} = \exp(1.21 + 0.379 * (\ln(\text{length}))^2)$	$\text{wt} = \exp(1.58 + 0.406 * (\ln(\text{length}))^2)$	1.06	1.18
Chinook salmon	$\text{wt} = 0.0113 * (\text{length}^3)$	$\text{wt} = 0.0264 * (\text{length}^3)$	1.12	1.38
Walleye (pickerel)	$\text{wt} = 0.0106 * (\text{length}^3)$	$\text{wt} = 0.0245 * (\text{length}^3)$	1.10	1.24

Appendix 5
Cleaning practices, parts eaten and cooking practices

Cleaning, Parts Eaten and Cooking for Great Lakes fish eaters

(percentages of participants eating and species)

Percentage of Great Lakes fish eaters using each cleaning practice 'usually' or 'sometimes' for each species

Cornwall

Species	No. of participants	Cut off and discard belly meat	Cut off and discard the fat from back	Cut off and discard the fat from the sides	Remove the skin	Puncture the skin	Cleaning score mean
Bass, largemouth	25	32.0	40.0	24.0	100.0	8.0	3.0
Bass, smallmouth	49	44.9	28.6	22.5	100.0	2.0	3.0
Brown bullhead	13	23.1	23.1	15.4	84.6	0.0	2.5
Perch, yellow	99	21.2	20.2	19.2	97.0	3.0	2.6
Pike, northern	21	47.6	33.3	23.8	81.0	4.8	2.9
Salmon, chinook	10	60.0	20.0	40.0	40.0	0.0	2.6
Salmon, coho	7	85.7	28.6	57.1	71.4	0.0	3.4
Salmon, unknown sp	6	50.0	50.0	50.0	33.3	0.0	2.8
Trout, lake	14	64.3	35.7	21.4	71.4	0.0	2.9
Trout, rainbow	12	41.7	25.0	16.7	50.0	0.0	2.3
Walleye	59	52.5	37.3	33.9	93.2	1.7	3.2

Mississauga

Species	No. of participants	Cut off and discard belly meat	Cut off and discard the fat from back	Cut off and discard the fat from the sides	Remove the skin	Puncture the skin	Cleaning score mean
Bass, largemouth	5	60.0	60.0	40.0	80.0	20.0	3.6
Bass, smallmouth	8	62.5	37.5	25.0	75.0	12.5	3.1
Perch, yellow	8	62.5	25.0	25.0	75.0	0.0	2.9
Pike, northern	10	70.0	20.0	20.0	80.0	10.0	3.0
Salmon, chinook	14	78.6	57.1	42.9	57.1	0.0	3.4
Salmon, coho	11	72.7	45.5	36.4	36.4	9.1	3.0
Salmon, unknown sp	3	100.0	33.3	33.3	66.7	0.0	3.3
Trout, lake	6	50.0	33.3	16.7	33.3	0.0	2.3
Trout, rainbow	20	75.0	35.0	30.0	60.0	10.0	3.1
Walleye	6	33.3	33.3	16.7	83.3	0.0	2.7

Percentage of Great Lakes fish eaters eating certain parts of each species

Cornwall

Species	No. of participants	Eat whole fish	Eat fillets	Eat steaks	Eat the skin	Parts eaten score mean
Bass, largemouth	25	8.0	100.0	0.0	4.0	3.9
Bass, smallmouth	49	4.1	98.0	2.0	4.1	3.9
Brown bullhead	13	61.5	30.8	0.0	0.0	2.7
Perch, yellow	99	2.0	99.0	1.0	1.0	4.0
Pike, northern	21	14.3	81.0	0.0	4.8	3.6
Salmon, chinook	10	30.0	40.0	50.0	30.0	3.3
Salmon, coho	7	0.0	57.1	57.1	14.3	4.0
Salmon, unknown sp	6	33.3	33.3	33.3	16.7	3.2
Trout, lake	14	21.4	78.6	7.1	7.1	3.6
Trout, rainbow	12	33.3	50.0	16.7	8.3	3.3
Walleye	59	1.7	89.8	3.4	0.0	3.9

Mississauga

Species	No. of participants	Eat whole fish	Eat fillets	Eat steaks	Eat the skin	Parts eaten score mean
Bass, largemouth	5	0.0	80.0	0.0	20.0	3.6
Bass, smallmouth	8	37.5	75.0	0.0	12.5	3.3
Perch, yellow	8	12.5	100.0	0.0	25.0	3.6
Pike, northern	10	30.0	80.0	0.0	10.0	3.4
Salmon, chinook	14	7.1	78.6	14.3	0.0	3.9
Salmon, coho	11	27.3	63.6	18.2	0.0	3.6
Salmon, unknown sp	3	0.0	66.7	33.3	0.0	4.0
Trout, lake	6	33.3	66.7	33.3	16.7	3.5
Trout, rainbow	20	25.0	55.0	25.0	10.0	3.5
Walleye	6	0.0	100.0	0.0	0.0	4.0

Percentage of Great Lakes fish eaters using each cooking practices for each species

Cornwall

Species	No. of participants	Deep fat fry or pan fry	Re-use frying fat	Bake, broil, grill or BBQ	Poach, boil, microwave or steam	Cooking score mean
Bass, largemouth	25	100.0	12.0	48.0	8.0	2.4
Bass, smallmouth	49	100.0	10.2	22.5	10.2	2.2
Brown bullhead	13	92.3	7.7	7.7	7.7	2.2
Perch, yellow	99	98.0	6.1	21.2	12.1	2.3
Pike, northern	21	81.0	4.8	33.3	19.1	2.7
Salmon, chinook	10	60.0	0.0	70.0	0.0	3.1
Salmon, coho	7	57.1	0.0	71.4	14.3	3.3
Salmon, unknown sp	6	33.3	0.0	50.0	0.0	3.2
Trout, lake	14	71.4	7.1	57.1	14.3	2.9
Trout, rainbow	12	50.0	0.0	66.7	8.3	3.3
Walleye	59	93.2	5.1	20.3	8.5	2.3

Mississauga

Species	No. of participants	Deep fat fry or pan fry	Re-use frying fat	Bake, broil, grill or BBQ	Poach, boil, microvave or steam	Cooking score mean
Bass, largemouth	5	100.0	0.0	80.0	20.0	3.0
Bass, smallmouth	8	87.5	0.0	37.5	12.5	2.6
Perch, yellow	8	87.5	0.0	25.0	12.5	2.5
Pike, northern	10	80.0	0.0	50.0	0.0	2.7
Salmon, chinook	14	50.0	0.0	57.1	7.1	3.1
Salmon, coho	11	18.2	0.0	72.7	9.1	3.6
Salmon, unknown sp	3	66.7	0.0	66.7	0.0	3.0
Trout, lake	6	33.3	0.0	100.0	0.0	3.7
Trout, rainbow	20	60.0	5.0	65.0	10.0	3.1
Walleye	6	100.0	0.0	0.0	0.0	2.0

APPENDIX 6

GREAT LAKES ANGLERS PILOT EXPOSURE ASSESSMENT STUDY

DESCRIPTION OF BIOLOGICAL SAMPLE COLLECTION PROCEDURES AND ANALYTICAL METHODS

Douglas Haines
Great Lakes Health Effects Program
Environmental Health Directorate
Health Protection Branch

April 25, 1995

File: D:\WPFILES\FISH\PROCEDUR.REP

TABLE OF CONTENTS

I. BIOLOGICAL SAMPLE COLLECTION, STORAGE, AND SHIPMENT 1
  I.A. Blood 1
  I.B. Urine 2
  I.C. Hair 2

II. ANALYTICAL METHODS 4
  II.A. PCBs, Organochlorine Pesticides, Dioxins and Furans 4
    II.A.i. PCBs and Organochlorine Pesticides in Plasma 4
    II.A.ii. PCDFs, PCDDs, and Coplanar PCBs in Plasma 8
  II.B. Metals 11
    II.B.i. Cadmium in Blood 11
    II.B.ii. Lead in Blood 13
    II.B.iii. Mercury in Blood 16
    II.B.iv. Total and Inorganic Mercury in Hair 21
  II.C. Lipids, Clinical Chemistry and Thyroid Function Test in Whole Blood 25
    II.C.i. Blood Lipids 25
      II.C.i.a. Cholesterol in Serum 25
      II.C.i.b. Free Cholesterol 28
      II.C.i.c. Phospholipids 31
      II.C.i.d. Triglycerides in Serum 34
    II.C.ii. Clinical Chemistry 37
      II.C.ii.a. Alanine Aminotransferase in Serum 37
      II.C.ii.b. Albumin in Serum 40
      II.C.ii.c. Alkaline Phosphatase in Serum 43
      II.C.ii.d. Aspartate Aminotransferase in Serum 46
      II.C.ii.e. Gamma Glutamyl Transferase in Serum 49
    II.C.iii. Thyroid Hormones and Thyroid Function 52
      II.C.iii.a. Free T4 52
      II.C.iii.b. Thyroid Stimulating Hormone (TSH) 55
      II.C.iii.c. Total T_3 61
  II.D. Subclinical Effects Markers 65
    II.D.i. Chromosomal Aberrations 65
    II.D.ii. D-glucaric Acid in Urine 67
    II.D.iii. Porphyrins in Urine 70
  II.E. Confounding Exposure Markers 73
    III.E.i. Cotinine in Urine 73

III. ANALYTICAL QUALITY CONTROL CHECKS 76
  A. Organochlorine Pesticides and PCB Analysis in Blood 76
  B. Metals Analysis in Blood and Hair 111

APPENDIX 1 Pre-Study Laboratory Audit: PCBs and Organochlorine Pesticides in Plasma 113

APPENDIX 2 Laboratory Quality Control for PCBs and Organochlorine Pesticides 116

APPENDIX 3 Disposition of Biological Samples 118

I. BIOLOGICAL SAMPLE COLLECTION, STORAGE, AND SHIPMENT

The biological samples collected from each participant in this study included blood, urine, and hair. All samples were collected by trained nurses during the field team's visit to the participant's homes. The materials and equipment necessary for sample collection were carried by the field team to each home they visited. Prior to the appointment, all vacutainers, vials, and bags used to collect and transport samples were appropriately labelled with the participant's serial number, initials, and date of sampling, as well as the type of sample collected and the type of analysis to be performed. The procedures followed to collect biological samples are described below.

I.A. Blood

Blood samples were collected, by venipuncture, from the antecubital vein of either the left or right arm of each participant while s/he was sitting. Venipuncture was performed immediately after the site was cleaned with alcohol using a 21 gauge disposable, multisample, stainless steel needle and a vacuumized tube system. Because different types of analyses were to be performed on blood samples (i.e. organochlorines, coplanar PCBs, clinical chemistry and lipid profiles, metals, and chromosomal aberrations), it was necessary to collect the blood in five separate vacutainers. The following order of sampling priority was assigned in the event that not all blood could be collected from a participant *1)* organochlorine pesticides, *2)* coplanar PCBs, *3)* clinical chemistry, lipid profiles, and thyroid hormones, *4)* metals, and *5)* chromosomal aberrations.

Organochlorine Pesticides: Blood was collected into a 10 mL lavender top, metal free vacutainer containing the anticoagulant EDTA (Becton Dickinson #6457). Immediately following the collection of all subsequent blood samples, this sample was centrifuged. Using a disposable glass pipette, the plasma was then transferred to a 7 mL vial with a teflon lined screw cap (Supelco #2-3248). Both the pipette and the vial had previously been washed by the analytical laboratory to prevent possible contamination. The plasma was transferred to the vials in order to avoid possible leaching of contaminants from the vacutainer's rubber stopper during shipment and storage. Once transferred, the plasma sample was then refrigerated, stored in a protective styrofoam mailer, and delivered to the laboratory within 48 hours in a shipping cooler with freezer packs. A specimen report form was filled out and accompanied the sample to its destination. Upon receipt by the laboratory, the samples were frozen at -15°C until analysed.

Coplanar PCBs: Identical collection and handling procedures as described for the Organocholorine pesticides were followed for the samples destined for coplanar PCB analysis. Upon receipt by the laboratory, the samples were frozen at -80°C until analysed.

Clinical Chemistry and Lipid Profiles: Blood was collected into a 9.5 mL, red top serum separator tube (SST) containing silica gel (Becton Dickinson #6510). Following the collection of all subsequent blood samples, this sample was centrifuged. The sample was then refrigerated, stored in a protective styrofoam mailer, and delivered to the laboratory within 48 hours in a shipping cooler with freezer packs. A specimen report form was filled out and accompanied the sample to its destination.

Metals: Blood was collected into a 7 mL royal blue top, metal free vacutainer containing the anticoagulant sodium heparin (Becton Dickinson #6527). Once filled, the tube was gently inverted 7 to 10 times to ensure complete mixing with the sodium heparin. The sample was then refrigerated, stored in a protective styrofoam mailer, and delivered to the laboratory within 48 hours in a shipping cooler with freezer packs. A specimen report form was filled out and accompanied the sample to its destination.

Chromosomal Aberrations: Blood was collected into a 10 mL green top, brand tube vacutainer containing the anticoagulant sodium heparin (Becton Dickinson #6480). Once filled, the tube was gently inverted 7 to 10 times to ensure complete mixing with the sodium heparin. The sample was then stored at room temperature in a protective styrofoam mailer and delivered to the laboratory within 48 hours in a shipping cooler with freezer packs. Caution was taken so that the styrofoam mailer with these samples did not come into contact with the freezer packs during shipment. This was necessary to avoid any large change in the sample's temperature which could affect blood cell viability. A specimen report form was filled out and accompanied the sample to its destination.

I.B. Urine

Two 100-mL all-plastic containers with leak proof screw caps were provided to each participant either on the day prior to the home visit, or on the day of the visit. Sodium carbonate, a bactericide and pH stabilizer had been added to one of the two containers. The participants were asked to collect their first morning void in the container with the sodium carbonate. They then stored the sample in their refrigerator in the brown paper bag supplied to protect the sample from light. The field team then retrieved the urine sample during their home visit or on the following day. At this time, approximately one-half of the sample (a minimum volume of 10 mL was required) was poured into the second all-plastic container. The urine in the container with sodium carbonate was destined for porphyrin analysis while the second container was for D-glucaric acid analysis. The samples were kept refrigerated and delivered to the laboratory within 48 hours in a shipping cooler with freezer packs. A specimen report form was filled out for each urine container and accompanied the sample to its destination.

I.C. Hair

The longest hairs were selected from the back of the head and a bundle of approximately five millimetres in diameter was isolated. A hemostat was used to fasten the selected hairs about two millimetres from the base of the scalp. The hair was then twisted about one full turn by rotating the hemostat. Using scissors, the hair was then cut as close as possible to the scalp. Care was then taken to ensure that the cut ends of the hair remained even during subsequent handling.

The hair sample was then transferred to a polyethylene bag (30 cm x 20 cm). The sample, while still fastened to the hemostat, was placed at the bottom of the bag. To prevent the sample from moving within the bag, the root end of the hair was stapled to the bag (two or three staples). Care was taken to keep the hairs even at the cut edge. The hemostat was carefully removed after the hair was stapled to the bag. Using a felt marker, a "P" (i.e. Proximal) was written on the bag where the hair had been cut, and a "D" (i.e. Distal)at the other end. The top of the bag was then folded over once and stapled closed. Further precautions for the preservation of the sample was not necessary. The hair sample was then delivered to the laboratory along with the blood samples in the shipping cooler. A specimen report form was filled out and accompanied the sample to its destination.

II. ANALYTICAL METHODS

II.A. PCBs, Organochlorine Pesticides, Dioxins and Furans

II.A.i. PCBs and Organochlorine Pesticides in Plasma

Target Compounds

The following PCBs and organochlorine pesticides were analysed from human plasma by gas chromatography (mass spectrography) using electron capture detectors:

PCBs: total PCBs as Aroclor 1260 and 13 PCB congeners: IUPAC Numbers 28, 52, 99, 101, 118, 128, 138, 153, 156, 170, 180, 183, and 187;

Organochlorine pestides: aldrin, dieldrin, β-hexachlorocyclohexane (β-HCH), p,p'-DDT, p,p'-DDE, α-chlordane, γ-chlordane, oxychlordane, cis-nonachlor, transnonachlor, hexachlorobenzene (HCB), heptachlor epoxide, mirex.

Summary of Analytical Method

The PCBs and organochlorine pesticides were extracted from plasma by a mixture of ammonium sulfate/ethanol/hexane (1/1/3 v/v/v). The extracts were concentrated and purified on two activated Florisil columns. Elution was carried out in two stages: the first fraction was eluted using a mixture of 35% methylene chloride in hexane and contained all target compounds except heptachlor epoxide and dieldrin; the second fraction was eluted using a mixture of 8% acetone in hexane and contained heptachlor epoxide and dieldrin. The extracts were analysed quantitatively and qualitatively on two capillary (Ultra 1 and Ultra 2) columns by gas chromatography (mass spectrography) using two electron capture detectors. Quantification was based on data from an Ultra 2 (HP-5) column. Identification was based on the retention times from both columns (Ultra 1 and Ultra 2).

Internal Standards

Each sample contained an internal standard for each of the two fractions. A PCB-198 internal standard was used for the first fraction and endrin was used as the internal standard for the second fraction. Prior to extraction, both of these internal standards were added to the plasma. These standards were used to correct for any recovery losses that may have occurred.

Calculation of results

The computerized work station calculated the area of each peak, where:

$$K = \frac{\text{Peak area of sample}}{\text{Peak area of internal standard}}$$

and

$$RF = \frac{5\ \mu g/L \text{ or } 0.5\ \mu g/L}{K}$$

The concentration of the target compound was then calculated as follows:

$$\text{concentration } (\mu g/L) = K \times RF$$

Using this calculation, all results were corrected for any recovery loss during the run.

Limits of Detection and Quantification

The analytical method's limit of detection and limit of quantification are described in the table below.

Compound	Limit of Quantification (μg/L)	Limit of Detection (μg/L)
Total PCB, expressed as Aroclor 1260	0.5	0.2
PCB congeners	0.05	0.02
Heptachlor epoxide and Dieldrin	0.2	0.1
β-HCH, and p,p'-DDT	0.1	0.03
Other Organochlorine pesticides	0.05	0.02

Quality Assurance and Quality Control

All glassware was cleaned and washed, and all reagents were prepared according to the laboratory's standard procedures. The following standards and sample spikes were included with each batch of samples analyzed:

- One standard (PCB congeners and organochlorine pesticides) at 0.5 µg/L, without extraction in hexane. This standard was injected at the beginning of the sequence and was used to check the sensitivity of the detectors;
- One standard (PCB congeners and organochlorine pesticides) at 0.5 µg/L, extracted in distilled water. This sample spike was used to calibrate the measurements of all target compounds except hexachlorobenzene and aldrin;
- One standard (PCB congeners and organochlorine pesticides) at 0.5 µg/L, extracted in plasma. This sample spike was used to calibrate the measurements of hexachlorobenzene and aldrin and to determine the percentage of recovery;
- One standard (Aroclor 1260) at 0.5 µg/L, extracted in distilled water was used to calibrate the measurement of total PCB.

The following two controls (Reference Materials), of known concentration, were analysed with each batch of samples analyzed:

- Serum NBS #1589 (1/10 dilution) containing the organochlorine target compounds (PCBs and organochlorine pesticides) at 0.4 µg/L;
- Normal human plasma Otw #2, from the Centre Hospitalier de l'Université Laval.

One control sample was injected at the start of the sequence just after calibration. The second control sample was injected at the end of the sequence. At every second batch of samples analyzed, a blind control was analysed. The results of the control samples were summarized in tabular form and on the laboratory's control chart. The maximum deviation accepted was ± 2σ.

References

1. Grant D.L. PCBs: Human and Environmental Hazards. Frank M. D'Itri; Michael A. Kamien. Butterworth Publishers, pp 383-387, 1984.
2. Ballschmitter K, Zell M. Analysis of polychlorinated biphenyls by glass capillary GC. *Anal. Chem.*, 302:20-31, 1980.

3. Lopez J.E.Q. Comparative study of clean-up and fractionation methods for the determination of organochlorine pesticides in lipids by gas chromatography. *J. of Chromatogr.* 591: 303-311, 1992.

4. Mes J. Organochlorine residues in adipose tissue of Canadians. *Bull. Environ. Contam. Toxicol.*, 45:681-688, 1990.

5. Norstrom R.J. Organochlorine contaminants in arctic marine food chains: Identification, graphical distribution, and temporal trends in polar bears. *Environ. Sci. Technol.*, 22(9), 1988.

II.A.ii. PCDFs, PCDDs, and Coplanar PCBs in Plasma

Sampling

The plasma sample in a glass tube was weighed and transferred with the aid of a few mL of water to a 250 mL Erlenmeyer flask. The original glass vessel was then dried and reweighed and the weight difference used as the whole or wet weight of sample.

Extraction

To the plasma sample in a 250 mL Erlenmeyer flask were added the internal standard stable isotopes used for quantification. These consisted of thirteen ^{13}C-PCDDs and ^{13}C-PCDFs, all but one (1,2,7,8-TCDF) with 2,3,4,8-substitution, and three ^{13}C-PCBs (IUPAC #s 77, 126, and 169) in amounts varying from 50 to 250 pg all in a small volume (10 to 20 μL) of toluene. Then in order, were added 50 mL of ethanol, 50 mL of saturated aqueous ammonium sulfate, and 100 mL of hexane. The mixture was then homogenized for 1 min with a mechanical homogenizer (Polytron) and transferred to a 250 mL separatory funnel. After phase separation which typically requires no centrifugation with small volumes of plasma samples, the lower aqueous and solid phases and the upper hexane phases were drawn off, and a further 100 mL of hexane was added to the lower aqueous and solid phases. The mixture was then rehomogenized/mixed in the separatory funnel with the homogenizer and the separated hexane phase combined with the first hexane extract. The combined hexane extracts were washed with 60 mL of water, and filtered through a funnel containing a little anhydrous sodium sulfate into a 250 mL round bottomed flask. The dried hexane extracts were concentrated on a rotary under vacuum to a few mL, and quantitatively transferred to a tared 50 mL flask. The extract was evaporated to a small volume in a rotary evaporator under vacuum and then to dryness under a stream of nitrogen. After the flask and its contents had attained constant weight, the weight difference was used as the lipid extractable amount of the plasma sample.

Cleanup

1. The lipid material was redissolved in about 150 mL of hexane in a 250 mL separatory funnel, and shaken with 20 mL portions of concentrated sulphuric acid which, after phase separation, were discarded. The addition, shaking and withdrawal of acid was repeated about five to seven times until the hexane extract was clear and pale yellow. The hexane extract was then washed with 20 mL water, 20 mL 1 % aqueous KOH, water, dried, and concentrated under vacuum to 1-2 mL.

2. The hexane extraxt was then passed through two columns arranged in tandem. The first column in a 10 mL disposable pipette contains, from top to bottom, separated portions of 40 % cesium silicate (0.5 g) and 40 % sulphuric acid (2.0 g) both adsorbed to silica gel. The second column was Florisil (1.5 g) activated at 140°C and not deactivated. The hexane extract was added to the first column and the eluent from this column allowed to flow directly into the Florisil column. The silicate column was then rinsed with a further 20 mL of hexane and, after all the hexane eluent from the first column had entered the Florisil column, the silicate column was removed. The Florisil column was then washed with 1 % dichloromethane in hexane (20 mL) and this fraction along with the 20 mL of hexane from

the top silica column were saved if measurement of all PCB congeners was required. The OCDFs/PCDDs/coplanar PCBs were eluted from the Florisil column with 50 mL of dichloromethane. The dichloromethane was taken just to dryness under vacuum and dissolved in 1-2 mL of hexane.

3. The carbon column consisted of 0.25 g of 18 % Carbopack C dispersed on Celite 545 (silica gel). The hexane extract from 2) above was added to the carbon column, and the column washed with hexane (2 mL), cyclohexane-dichloromethane (1:1; 1 mL), and the planar analytes eluted with 15 mL of toluene. After removal of the toluene solvent by a combination of rotary evaporation in vacuum and under nitrogen, the final extract was dissolved in 3 to 5 μL of toluene containing 1,3,6,9-TCDD and 1,2,3,4,6,8-HxCDD, both at concentrations of 5 pg/μL. The latter two standards were used to monitor recovery of the isotope internal standards.

Mass Spectrometry

A Kratos Concept double focussing sector mass spectrometer (MS) was used in the electron impact ionization mode at 40 to 70 eV and at high (10K) mass resolution. The MS was coupled directly via a heated interface to a Hewlett Packard 5890 gas chromatograph (GC) equipped with a 30 m, 0.25 mm id, 250 μ thickness, bonded phase non-polar DB-5 methyl silicone fused silica capillary column. The vector gas was helium at 30-45 cm/sec and injection by the solute focussing on-column technique. Multigroup selected ion monitoring of appropriate masses was controlled by a Data General data system switching from homologue tetra- up to octa- at specified times as determined by an established window of GC retention times. The descriptor ions used consisted of six discrete groups. The first of these monitors trichlorobiphenyl (TCB; basically the # 77 congener), and small portions of the TCDF and TCDD elutions and, along with the MS reference (perfluorokerosene) and lock masses, consisted of a total of 14 ions. The second group contained the ions for pentachlorobiphenyls (PnCB; congener # 126), and the major portion of the TCDF and TCDD windows with the ion switching occurring just after the elution of PCB # 77 and 1,3,6,8-TCDF and before the elution of 2,4,6,8-TCDF and 1,3,6,8-TCDD. The third group monitors hexachlorobiphenyls (HxCB; congener # 169), all pentachloro dibenzofurans (PnCDFs), and pentachloro dibenzodioxins (PnCDDs; 14 ions), the fourth hexachloro dibenzofurans (HxCDFs) and HxCDDs (hexachloro dibenzodioxins: 10 ions), the fifth heptachloro dibenzofurans (HpCDFs) and heptachloro dibenzodioxins (HpCDDs: 10 ions), and the sixth and last octochloro dibenzofurans (OCDF) and octochloro dibenzodioxins (OCDD; 8 ions). The ions monitored for the PCDDs/PCDFs/coPCBs were the two most abundant (100 % response) molecular ions based on chlorine for both the native carbon-12 and the stable isotope carbon-13 isotopomers. For example, for TCDD, the ions chosen were m/z 319.8965 (M+; 78 %) and 321.8936 (M+2; 100 %) for ^{12}C and m/z 331.9368 (M+; 78 %) and 333.9338 (M+2; 100 %) for ^{13}C. An exception to these selections was the ions for HxCB where the M+4 ion (81 %) and M+6 (35 %) ions were monitored in order to minimize overlap of the response of # 169 with the ions for ^{13}C-1,2,3,7,8-PnCDD, both of which elute at about the same time.

Quantification of the response from the normal (native) and stable isotope ions from the MS for an unknown sample extract was carried out with a single injection by the internal standard method using isotope dilution and relative response factors (RRF). The RRFs were obtained from the MS by a standard curve constructed from a single set of calibration standards. These consisted of eight solutions containing : (a) the isotopically labelled PCDDs/PCDFs (5 pg/μL) and PCBs (10 pg/μL) identical in congener to those in the sample spiking mixture added to the unknown sample at the beginning of analysis and (b) various concentrations of the native PCDDs/PCDFs/coPCBs in the range 0.1 to 25 pg/μL. These native analytes consisted of most of the 2,3,7,8-substituted congeners, the first and last eluting isomer from each homologue group, other congeners readily separated on the non-polar GC column and the three coplanar PCBs. The final concentration of all analytes in the sample was calculated by the ratio of the response of the native to the isotope ion, multiplied by the amount of isotope added at the beginning, divided by both the RRF and the weight of the sample. Concentrations were usually expressed in ng/kg (parts per trillion).

$$\text{Conc} = \frac{Rn}{R_{is}} \cdot \frac{Q_{is}}{RRF} \cdot \frac{1}{W}$$

Quality Assurance and Quality Control

Samples were usually analysed in batches of 9 to 12. One of these was a laboratory reagent blank to check for contamination during the sample preparation. Another was a QC sample of similar matrix as the unknowns an aliquot of which was analysed periodically to assess precision and control of the analytical procedure. Isotope ratios based on chlorine for unknown sample extracts must have been within 15 % of those found from standards. Standards were also injected several times daily. These, quantified as unknowns using the standard curve, must have been within 20 % of their nominal value. Blank samples usually contained less than one pg total of any congener except for OCDD which was less than 10 pg and PCB # 77 which was about 20 pg. A portion of about 10 % of all unknown samples were confirmed by reinjection on a polar cyanopropyl fused silica capillary column containing CP-Sil 88 at high mass (10K) resolution. This additional step identified more fully the isomer present.

Limit of Detection

The method's limit of detection (L.O.D.) varied with the volume of plasma analysed.

References

Ryan J.J., Lau B.P.-Y, Boyle M.J. Dioxin-like compounds in human blood. Biological Mass Spectrometry: Present and Future. Edited by Matsuo T., Caprioli R.M., Gross M.L. and Seyama Y. John Wiley & Sons Ltd., pp. 583-602, 1994.

Ryan J.J., Hsu C.-C., Boyle M.J., Guo Y.-L. L. Blood serum levels of PCDFs and PCBs in Yu-cheng children peri-natally exposed to a toxic rice oil. *Chemosphere*, 29(6) 1263-1278, 1994.

II.B. Metals

II.B.i. Cadmium in Blood

The cadmium concentrations in the blood of the participants were determined by graphite furnace atomic absorption spectrophotometry (GFAAS), using Zeeman background correction.

Materials and Equipment

Zeeman 4100 ZL
Liquid Handler (Gilson 212B)
Centrifuge
1.5 mL centrifuge microtubes
1mL and 10 mL RAININ pipettes
Gilson 250 µL Micro Man pipette

Reagents

Argon (UHP)
Nitric acid (Ultrex II, J.T. Baker)
Water (Deionized)

Reagent Preparation

Nitric acid (Ultrex II) 1.6M

50 mL of HNO_3 was added to a 500 mL volumetric flask which contained deionized water. The flask was then filled with deionized water to complete the volume.

Standards and Controls

Blood samples, of known cadmium concentrations, from l'Université Laval were used as standards and controls. Some of the blood samples obtained from the above were designated as standards and others were designated as controls in each batch run. Those designated as standards were used in the preparation of standard curves in each batch run. These samples were not used for both functions (standards and controls) in the same batch run. See the Quality Assurance and Quality Control section below for the use of control samples.

Clean Procedures

All flasks, pipette tips and disposable material were rinsed 2-3 times with nitric acid (10% - 20%) and rinsed again 2-3 times with deionized water. All glassware was washed with nitric acid (20%) and rinsed 2-3 times with deionized water.

Analytical Procedure

The vacutainers containing the blood was placed on a mixer for about 15 minutes. Gentle mixing of the blood was necessary in order to prevent air bubbles from forming in the sample. Using a 10 mL RAININ pipette, 600 µL of HNO_3 (Ultrex II) 1.6 M was pipetted into the 1.5 mL centrifuge microtubes. With the Gilson 250 µL Micro Man pipette, 200 µL of blood was then added to the nitric acid in the microtube which was immediately closed and vortexed. These dilutions were then centrifuged for 10 minutes. The supernatent was then transferred to the Liquid-Handler 212B autosampler sample cups (0.5 mL) using a 1 mL RAININ pipette. 10 µL of supernatent was injected into the graphite furnace of the 4100 ZL spectrophotometer. The blood cadmium concentration was then automatically calculated by the instrument (4100 ZL), using the calibration curve.

Limits of Detection and Quantification

The method's limit of detection (L.O.D.) was 0.005 µg/100 mL, and the limit of quantification (L.O.Q.) was 0.02 µg/100 mL.

Quality Assurance and Quality Control

A minimum of six control samples of known cadmium concentrations were included with each batch of twelve samples. Three blank samples were also included in each batch of samples run. All samples were analysed in duplicate. If the values of the control samples run were ± 10% from the target, all samples in the batch run were rejected and reanalyzed.

References

1. Knowles M. The determination of lead and cadmium in blood and manganese and aluminum in serum. *Varian Instruments at Work*, AA-76, 1987.
2. Dosage du cadmium sanguin pour four au graphite # 5000. Centre de Toxicologie du Quebéc, Le Centre Hospitalier de l'Université Laval, Québec.

II.B.ii. Lead in Blood

The lead concentrations in the blood of the participants was determined by graphite furnace atomic absorption spectrophotometry (GFAAS), using Zeeman background correction.

Equipment

Zeeman 4100 ZL
Liquid Handler (Gilson 212B)

Reagents

Argon (UHP)
Molybdenum and tungsten solution (1000 ppm), Spex Industries, Inc.
Triton-X (Kodak)
Water (Deionized)

Reagent Preparation

Triton-X 2.5% (w/v)

25 gm of Triton-X was added to a 1000 mL volumetric flask which was then filled with deionized water to complete to volume.

Triton-X 0.25% (v/v)

50 mL of the above Triton-X (2.5% p/v) solution was added to a 500 mL volumetric flask which was then filled with deionized water to complete to volume.

Standards and Controls

Blood samples, of known lead concentrations, from l'Université Laval and/or Ontario Ministry of Labour and/or American College of Pathologists were used as standards and controls. Some of the blood samples obtained from the above were designated as standards and others were designated as controls in each batch run. Those designated as standards were used in the preparation of standard curves in each batch run. These samples were not used for both functions (standards and controls) in the same batch run. See the Quality Assurance and Quality Control section below for the use of control samples.

Clean Procedures

All flasks, pipette tips and disposable material were rinsed 2-3 times with nitric acid (10% - 20%) and rinsed again 2-3 times with deionized water. All glassware was washed with nitric acid (20%) and rinsed 2-3 times with deionized water.

Analytical Procedure

The vacutainers (Becton Dickinson #6527) containing the blood was placed on a mixer for about 15 minutes. Approximately one mL of molybdenum and tungsten solution was added to the Triton-X 0.25% and to the blanks (deionized water) to ensure that the robot's metal detector was functioning properly. 30 µL of blood and 375 µL of Triton-X 0.25% was transferred to the sample cups (0.5 mL) using the Liquid-Handler 212B. The robot's PBSANG and PBTRAY programs were used. 10 µL of diluted blood was injected into the graphite furnace of the 4100 ZL spectrophotometer.

Calculation of Results

The blood lead concentration was automatically calculated by the instrument (4100 ZL), using the calibration curve generated from the standard blood samples. Each vacutainer sample was analyzed in duplicate and the mean concentration of the two sample preparations was reported as the final result.

Limits of Detection and Quantification

The method's limit of detection (L.O.D.) was 0.04 µg/100 mL, and the limit of quantification (L.O.Q.) was 0.5 µg/100 mL.

Quality Assurance and Quality Control

A minimum of six control samples of known lead concentrations were included with each batch of twelve samples. Three blank samples were also included in each batch of samples run. All samples were analysed in duplicate. If the values of the control samples run were ± 10% from the target, all samples in the batch run were rejected and reanalyzed.

References

1. Subramanian K.S. Determination of lead in blood by graphite furnace atomic absorption spectrometry - A critique. *The Science of the Total Environment*, 89: 237-250, 1989.

2. Miller D.T., et al. Determination of lead in blood using electrothermal atomisation atomic absorption spectrometry with L'vov platform and matrix modifier. *Analyst*, Vol 112, 1987.

3. Wang S.T., et al. Microsampling technique and determination of blood lead by Zeeman atomic absorption spectrometry. *The Science of the Total Environment*, 71: 37-43, 1988.

II.B.iii. Mercury in Blood

The total and inorganic mercury concentrations in the blood of the participants was determined by cold vapour atomic absorption spectrophotometry (CVAAS). An automated system was used to determine the total mercury content while a manual system was used to determine the inorganic mercury content.

Materials and Equipment

Scintillation vials (Kimble, 20 mL)
1 mL, 2 mL, and 10 mL dispensers
1 mL and 10 mL RAININ pipettes
Gilson 250 µL Micro Man pipette
Corning (PC-100) hot plate

Automated System (custom designed):

Mercury Monitor U.V., Models 1255 and 1235 using a 254 nm mercury lamp

Digitec Voltmeter, Model 2110 Multimeter (Megatronix Inc.)

Reaction apparatus including a reaction cell (non-commercial), Two 25 mL traps ("Impinger") with isotherm ("Dewar") flasks used as ice baths

Pumps and computers

Manual System:

In the case of the manual system, one non-commercial 20 cm reaction cell fitted with a funnel, a debimeter, and a carbon trap were used with a mercury monitor, a voltmeter, and a pump as described above.

Reagents

Cadmium chloride (Johnson Matthey Electronics)
L-Cysteine (Sigma)
Ethanol (Mallinckrodt)
(Ethylenedinitrilo) tetraacetic acid (EDTA), Disodium Salt, Dihydrate (J.T. Baker)
Mercuric chloride (Fisher)
n-Octanol (Anachemia)
Sodium chloride (J.T. Baker)

Sodium hydroxide (J.T. Baker)
Standard Materials (Blood Samples of known concentration from Université Laval)
Stannous chloride (J.T. Baker)
Sulphuric acid (Anachemia)
Water (Deionized)

Reagent Preparation

1. Anti-foaming Solution:

   1 mL of octanol was mixed with 9 mL of ethanol.

2. Sulphuric Acid/Sodium Chloride:

   In each of three Erlenmeyer flasks (4 L), 35 gm of sodium chloride was dissolved in 3 L of deionized water. Then 440 mL of concentrated sulphuric acid was added to the sodium chloride water mixture. The volume in each flask was then brought to 3500 mL with deionized water.

3. Reducing Solution ($SnCl_2$ + $CdCl_2$) -For Total Mercury

   In a 6 L Erlenmeyer flask, 40 gm of stannous chloride, 10 gm of cadmium chloride, and 4 gm of L-Cysteine were dissolved in 2000 mL of the sulphuric acid/sodium chloride solution prepared as in 2. above.

4. Sodium Hydroxide (45% w/v) - For Total Mercury

   In a 2 L Erlenmeyer flask, 810 gm of sodium hydroxide were dissolved in 1800 mL of deionized water.

5. Sodium Hydroxide (35% w/v)

   In a 6 L Erlenmeyer flask, 1820 gm of sodium hydroxide was dissolved in 5200 mL of deionized water.

6. Stannous Chloride ($SnCl_2$) -For Inorganic Mercury

   In a 4 L Erlenmeyer flask, 40 gm of stannous chloride and 4 g of L-Cysteine were dissolved in 2000 mL of the sulphuric acid/sodium chloride solution prepared as in 2. above.

7. Sulphuric Acid (5% v/v)

In a 1 L Erlenmeyer flask, 50 mL of concentrated sulphuric acid was added to 950 mL of deionized water.

8. L-Cysteine (1% w/v)

In a glass bottle, 3 gm of L-Cysteine was dissolved in 300 mL of deionized water.

9. Sodium Chloride (NaCl, 1% w/v)

In a glass bottle, 25 gm of sodium chloride was dissolved in 2500 mL of deionized water.

Clean Procedures

All glassware was washed with nitric acid (20%) and rinsed 2-3 times with deionized water.

Preparation of Standards (Inorganic Mercury)

500 ppm Hg^{+2} (Pre-stock)

In a volumetric flask, 0.33835 gm of mercuric chloride ($HgCl_2$) was dissolved in sulphuric acid (5%) and the volume was completed to 500 mL. This solution is stable for approximately 6 months if stored in a refrigerator.

500 ppb Hg^{+2} (Stock)

1 mL of the above 500 ppm Hg^{+2} (Pre-stock) solution, 9 gm of sodium chloride, 0.7545 gm of EDTA, and 0.1 gm of L-Cysteine were dissolved with deionized water and the volume was completed to 1 L. This solution is stable for approximately 6 months if stored in a refrigerator.

Working Solutions (10, 20, 30 ppb Hg^{+2})

In three different 200 mL volumetric flasks, 4, 8, and 12 mL of the above 500 ppb Hg^{+2} (Stock) solution were diluted with deionized water. These working solutions were prepared daily.

#1 Spike Solution (5 ppb Hg^{2+})

5 mL of a blood pool were added to 5 mL of the working solution (10 ppb Hg^{2+}).

#2 Spike Solution (10 ppb Hg^{2+})

5 mL of a blood pool were added to 5 mL of the working solution (20 ppb Hg^{2+}).

#3 Spike Solution (15 ppb Hg^{2+})

5 mL of a blood pool were added to 5 mL of the working solution (30 ppb Hg^{2+}).

Analytical Procedure

Total Mercury Analysis

For the analysis of total mercury in blood, the sample digestion was done manually and the analysis was carried out on the automated system which calculated and recorded the area of the peak, using an integrator. The results obtained from the standards were used to generate a calibration curve, using an Olivetti M386 or M290 computer.

Using a Gilson M-250 pipette, 1 mL of blood was pipetted into a 20 mL scintillation vial (Kimble). With dispensettes (Brinkmann), 0.5 mL of L-Cysteine (1%, solution 8) and 1 mL of sodium hydroxide (45%, solution 4) were added to the blood sample. The sample was then digested for about five minutes on a hot plate (Corning) which was adjusted to position 2, until a homogeneous sample was obtained. Caution was taken not to boil the sample. After gently shaking the contents of the vial, 2.5 mL of the sodium chloride solution (solution 9) was added and the vial was removed from the hot plate and prepared for analysis. 1 mL of the digested sample was inserted into the reaction cell. 5 mL of the reducing solution ($SnCl_2$ + $CdCl_2$, solution 3) and 5 mL of sodium hydroxide (35%, solution 5) were simultaneously inserted into the reaction cell. 50 µL of the anti-foaming solution (solution 1) were manually added to the reaction cell.

Inorganic Mercury Analysis

Inorganic mercury analysis was conducted without blood digestion, on the manual system. Using a Gibson M-250 pipette, 200 µL of blood was pipetted into the reaction cell. 50 µL of the anti-foaming solution (solution 1) was added with a pipette. 5 mL of stannous chloride (solution 6) and 5 mL of sodium hydroxide (35%, solution 5) were added with dispensettes. The reaction cell was connected immediately to the vacuum-monitor system. The results obtained from the spike solutions were used to generate a calibration curve, using an Olivetti M386 or M290 computer.

Calculation of Results

The calibration curve was generated from the standards (Total Mercury) (Université Laval) and spike solutions (Inorganic Mercury) using the Olivetti M386 or M290 computer. The area of the peaks and their corresponding concentrations were manually entered into the computer software which then calculated the results.

Limits of Detection and Quantification

The method's limit of detection (L.O.D.) was 0.4 ppb (0.04 μg/100 mL), and the limit of quantification was 2 ppb (0.2 μg/100 mL).

Quality Assurance and Quality Control

A minimum of one control sample of known mercury concentration was included with each batch of samples. Three blank samples were also included in each batch of samples. All samples were run in duplicate. If the values of the control samples run were ± 10% from the target, all samples in the batch run were rejected and reanalyzed.

References

1. Farant J.P., et al. Improved cold-vapor atomic absorption technique for the microdetermination of total and inorganic mercury in biological samples. *Journal of Analytical Toxicology*, vol.5, January/February, 1981.

II.B.iv. Total and Inorganic Mercury in Hair

The hair samples were analysed for total and inorganic mercury. The organic (methyl) mercury content was then estimated by subtracting inorganic from total mercury content.

Apparatus

Mercury Automated System - Customized;
Mercury Monitor - U.V.Monitor Model 1235 and 1255 (LDC) using a mercury lamp set at 254 nm;
Voltmeter-Digitec Model 2110 Multimeter (Megatronix Ltd.);
Reaction Apparatus - Reaction Cell
        25 mL Impinger
        Dewar Flask (Used as Ice Bath)
        Pump;
    Brinkman Dispensette 1.0, 2.0, 5.0 and 10.0 mL;
    Electronic Pipets Rainin 10.0 and 1.0 mL;
    Scintillation Vials;
    Corning Hot Plate - PC-100.

Reagents and Standards

1% w/v L-Cysteine — Solution 1
1% w/v NaCl — Solution 2
45% w/v NaOH — Solution 3
35% w/v NaOH (2L per box) — Solution 4
4.5 N H_2SO_4/1% w/v NaCl = — Solution 5
    440 mL concentrated H_2SO_4
    35 gm NaCl
    Dissolved and diluted to a volume of 3500 mL with demineralized water.
$CdCl_2/SnCl_2$ = — Solution 6
        20.0 g $SnCl_2 \cdot 7\ H_2O$
        5.0 g $CdCl_{27}$
        2.0 g L-Cysteine
    Dissolved in 1000 mL of 4.5 N H_2SO_4/1% NaCl which is solution 5 (1 L per box of 90 samples).
$SnCl_2$ = — Solution 7
        20.0 g $SnCl_2 \cdot 7H_2O$
        2.0 g L-Cysteine
    Dissolved in 1000 mL of 4.5 N H_2SO_4 / 1% NaCl which is solution 5 (1 L per box of 90 samples).
5% w/v H_2SO4 — Solution 8

Cleaning Solution = Solution 9

3.5 L of 4.5 N H_2SO_4 in a white jug (20 L). The jug was then filled to top with distilled water.

NOTE: All reagents were prepared with ultra- filtered, Millipore, Super-Q, demineralized water.

Hair Washing

Normally the hair was **not** washed but if required this procedure was followed. All the hair in the sample was aligned at the scalp and was well tied at three or more places along its length. The hair was then placed in a beaker containing detergent made of 1 mL Triton X-100, 100 mL demineralized water, and 2 mL 1% L-Cysteine (Solution 1) and shaken for 15 minutes. After shaking, the hair was rinsed with demineralized water and the wash and rinse procedure was repeated one mor time. Once washed, the hair sample was air dried on a mercury-free paper towel for 24 hours.

Preparation of Hair Sample

The hair samples were collected and cut into one-centimetre segments. The 10 ± 2 mg hair segments were weighed. Accurate weighing was a very critical procedure and was the main source of error made in the hair analysis procedure. Analysis was performed on centimetre segments 1, 2, 3, 5, 7, 9, ... according to the length of the hair.

Digestion

To each vial containing a centimetre segment of hair was added 1 mL of 1% L-Cysteine (Solution 1) and 2 mL of 45% NaOH (Solution 3). The sample was heated until almost boiling (90°C) (10 min) while the vial was gently swirl to dissolve the hair sample (excepting the hair pigment). Solution 2 (1% NaCl) was then added to the vial to a volume of 10 mL and appropriate aliquots were then used for total and/or inorganic mercury analysis. A 1.0 mL aliquot of the digest solution was normally used for analysis.

Sample analysis

1. One (1.0 mL) of the digest solution was placed in the reaction vessel. Added to this in sequence were:

   a. 5 mL of reducing agent (solution 6 ou 7)[*]
   b. 5 mL of 35% NaOH (Solution 4)

   [*] These were two reducing agents used:
      i. For **Total Mercury** - Solution 6, $CdCl_2/SnCl_2$ mix
      ii. For **Inorganic Mercury** - Solution 7, $SnCl_2$ mix

2. The above mixture was integrated for 40 seconds.
3. The peak area was then recorded and saved on disk.

Limits of Detection and Quantification

The method's limit of detection (L.O.D.) was 0.5 ppb and the limit of quantification was 0.5 ppm (500 ppb).

Quality Assurance and Quality Control

Mercury Standards

Pre-stock - 500 $\mu g\ Hg^{+2}/mL$ (Good for 6-12 months if refrigerated)

0.33835 g $HgCl_2$ dissolved in 5% H_2SO_4 to a volume of 500 mL.

Stock - 500 ng Hg^{+2}/mL (Good for 1-2 months if refrigerated)

1.0 mL of Pre-stock with 9.0 g of NaCl and 0.7545 g EDTA and 0.1 g L-Cysteine dissolved in water to a volume of 1 Litre.

Working Solution (prepared daily) - 10 ng Hg^{+2}/mL

4 mL of Stock diluted to 200 mL in water.

A sequence of 20 bottles were analysed. For each set of 14 samples, one standard, three blanks, one low pool and one high pool were run. The **low pool** was a mixture of digested hair samples previously analysed to determine low concentration. The **high pool** was a mixture of digested hair samples previously analysed to determine high concentration. An analytical run would be rejected if the precision of a day's analytical results for the standard (10 ppm) and the High Pool (value varies according to sample availability) was greater than 5% RSD.

Calculations for Mercury Concentrations in Hair

Robot Automatic System Calculation

$$\text{Mercury Concentration} = \frac{(S-B) * Q * V * Df}{(STD-B) * A * W}$$

Where S = Sample Value (Peak Area)
B = Blank Value (Peak Area)
Q = Quantity (ng) of Mercury used to obtain STD
Df = Dilution Factor (if necessary)
STD = Standard Value (Peak Area)
A = Sample Volume used in mL (1 mL for automatic system)
W = Weight of hair prior to digestion (mg)
V = Final Sample Volume After Digestion (Usually 10 mL for automatic system)

Reference

1. Farant, J.P., et al, Improved Cold-Vapor Atomic Absorption Technique for the Microdetermination of Total and Inorganic Mercury in Biological Samples, *Journal of Analytical Toxicology*, Vol. 5, January/February, 1981.

II.C. Lipids, Clinical Chemistry and Thyroid Function Test in Whole Blood

II.C.i. Blood Lipids

II.C.i.a. Cholesterol in Serum

Summary/Automated Colorimetry

Total cholesterol was determined colorimetrically using cholesterol esterase and cholesterol oxidase with the resulting peroxide coupled to the formation of a red quinoid dye. A 3 µL patient sample was mixed with buffered cholesterol esterase and oxidase and 4-aminophenazone as a single reagent system. The resulting color was measured bichromatically at 505 and 700 nm:

$$\text{Cholesterol Esters} + H_2O \xrightarrow{\substack{\text{CHOLESTEROL}\\ \text{ESTERASE}}} \text{Cholesterol} + \text{Fatty Acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\substack{\text{CHOLESTEROL}\\ \text{OXIDASE}}} \Delta^4\text{ - Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{4-Aminophenazone} + \text{Phenol} \xrightarrow{\text{POD}} \text{4-(p-benzoquinone)phenazone}$$

Linearity: 0-18.8 mmol/L
Sensitivity: 0.1 mmol/L
Precision: 1.5 per cent CV at 5.5 mmol/L

Sample

Serum: SST.

Interference:

Hemolysis - no effect from mild to moderate (+ to ++); severely hemolysed (+++) samples were not run or reported.

Bilirubin - no effect up to 340 µmol/L.

Lipemia - no effect from light to moderate lipemia (up to 8.8 mmol/L triglyceride). Grossly lipemic samples could be cleared using the Beckman Airfuge (10 minutes at 30 psi).

Reagents

R1 - Cholesterol Reagent - BMC cat. no. 1127578/600 ml: tris buffer, 100 mmol/L, pH 7.7;
Mg++, 50 mmol/L;
4-amino-phenazone, 1 mmol/L;
sodium cholate, 10 mmol/L;
phenol, 6 mmol/L;
3,4-dichlorophenol, 4 mmol/L;
cholesterol esterase, 400 U/L;
cholesterol oxidase, 250 U/L and peroxidase, 200 U/L.

The bottle contents were dissolved in 600 ml of deionized water. The reconstituted reagent was stable for 4 weeks when stored at 4°C.

Procedure

1. It was ensured that the proper system parameters had been loaded into both Hitachi instruments using SYS 9, 14 ie:

(1) Assay code	ENDP-20
(2) Sample volume (µL)	3
(3) R1 volume (µL)	300
(4) R2 volume (µL)	0
(5) Wavelength 1 (nm)	505
Wavelength 2 (nm)	700
(7) Calibration 01 Saline	0
02 Cal	See defined value
(8) Equation	1
(9) Unit factor	See defined value

2. If fresh reagent had been added, the volume was adjusted using RTN-1 and a full prime using PRIMING DISC in RTN-1 was done, followed by MTN-2.

3. Calibrators using RTN-2 and QC's and controls using RTN-5 were requested.

4. A Q listing was prepared in the Cerner LIS system under WQM, work-center 110, site 110, matching the 3 digit stamped sequence number against the 7 digit requisition number.

5. A minimum of 200 µL of patient sample was transferred to the pre-numbered sample cup and barcode reader tube bearing the same stamped sequence number as the patient specimen.

6. The patient sample cup/barcode tube was loaded into any open position on a sampling carousel, with appropriate QC's (2) and controls (2) in positions 1, 2, 20 and 40 as required.

7. The carousel was loaded into one of the Hitachi analyzers, and RUN was typed in from the home position of any screen indicating tray and cup number (RUN X XX ENTER). The barcode reader was monitored from RTN-3.

8. After the patient results were printed, a copy of the patient requisition was stapled to the report; patient ID, sequence number and test selection were verified. The patient results were checked for codes, linearity limits or non-conforming results.

9. Samples above the linearity limit were diluted 1:2 with saline and re-run; the analyzer results were multiplied by 3.

10. The report was initialled before sending for computer entry.

Reference Range

Serum/plasma:		moderate risk	high risk
	20-29 yrs	> 4.7 mmol/L	> 5.7 mmol/L
	> 30 yrs	> 5.2 mmol/L	> 6.2 mmol/L

Clinical Significance

Decreased Serum Cholesterol	Increased Serum Cholesterol
α-Lipoprotein deficiency	Hyperlipoproteinemias
Hepatic neoplasms	Nephrotic syndrome
Malnutrition	Chronic renal failure
Megaloblastic anemia	Diabetes
Chronic obstructive lung disease	Alcoholism

References

1. BMC Hitachi 737 Operating Reference Manual

2. Tietz NW, Fundamentals of Clinical Chemistry, 1987, pp 473-5

II.C.i.b. Free Cholesterol

Summary

Free cholesterol levels were determined by oxidation of free cholesterol with cholesterol oxidase to form cholestenone and hydrogen peroxide (cholesterol esters do not react with cholesterol oxidase). The hydrogen peroxide was then coupled to aminophenazone to form a dye complex which was monitored at 500 nm:

$$\text{cholesterol} + O_2 \xrightarrow{\text{cholesterol oxidase}} \text{cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{4-(p-benzoquinone-monoimino)phenazone} + 4H_2O$$

Sensitivity: 0.2 mmol/L
Linearity: 0-7.76 mmol/L
Precision: 4%

Sample

Serum, heparinized plasma or EDTA plasma.

Reagents

BMC Free Cholesterol test kit, cat. no. 310328

1. Buffer (Potassium phosphate), phenol, methanol.

2. Buffer (Potassium phosphate), 4-aminophenazone, methanol, hydroxypolyethoxydodecane.

3. Cholesterol oxidase, peroxidase.

4. Cholesterol standards (1-6). BMC Preciset, cat. no. 125512.

Undiluted solutions were used. The solutions were stable up to the expiry date specified when stored at +2 - +8°C.

Procedure

1. The cholesterol reagent was prepared: 0.5 mL of sol. 3 was pipetted into bottle 2 and the entire contents of one bottle 1 was added, or the stock solution was prepared according to the following table:

Sol'n 1 ml	Sol'n 2 ml	Sol'n 3 ml
5	5	0.05
10	10	0.10
20	20	0.20
40	40	0.40

2. 50 µL of the QC and patient samples were pipetted into the appropriate sample cups and the sample rack was placed on the Cobas.

3. With power on, TEST NR 15-FREE CHOLESTEROL was entered.

4. Using STEP PAR, the following parameters were checked. The concentrations of the standards were checked and should have been the same as on each standard bottle that was being used. There were six standards, but only 3 (1st, 3rd, 5th standards) were used.

Parameter Listing

1. Units	mmol/L
2. Calculation factor	0
3. Standard 1 conc.	1.29
4. Standard 2 conc.	3.88
5. Standard 3 conc.	7.76
6. Limit	7.76
7. Temperature (Deg. C)	37.0
8. Type of analysis	5
9. Wavelength (nm)	500
10. Sample volume (µL)	05
11. Diluent volume (µL)	10
12. Reagent volume (µL)	350
13. Incubation time (sec)	0
14. Start reagent volume (µL)	0
15. Time of first reading (sec)	.5
16. Time interval (sec)	60
17. Number of readings	07
18. Blanking mode	1
19. Printout mode	1

5. The cholesterol solution was poured into the primary well of the reagent tray and 100 µL of appropriate standard was pipetted into standard wells.

6. The tray cover was attached placed on Cobas.

7. The photometer arm was lifted and the cuvette ring was placed on the rotor. Position The clamping ring was positioned and the photometer arm was lowered.

8. The plexiglass cover was closed. Start was pressed and the pipettor arms were observed for proper operation action.

9. Upon completion of analysis, the printed results were removed, the QC results were checked and logged, and flagged and over-limit results were checked for.

10. If any result exceeded the highest standard concentration, the sample was diluted appropriately with saline and reassayed. The result was multiplied by the dilution factor.

11. Results were recorded on the worklist and the data were then entered.

Reference Interval

22-30% of total cholesterol.

Clinical Significance

Free cholesterol or unesterified cholesterol molecules are found on the outside of lipoprotein molecules. The other components of the lipoprotein molecule include non-polar esterified cholesterol, phospholipids, triglycerides and apoproteins. The proportion of each of these components varies from one lipoprotein molecule to next (i.e. HDL, VLDL, LDL, chylomicrons). Free cholesterol levels represent 22-30% of total cholesterol in normal individuals. Free cholesterol levels are used in research to evaluate lipid metabolism.

References

1. Boehringer Mannheim GmbH. Test-Combination Free Cholesterol. Enzymatic colorimetric method, Cat. No. 310328, June, 1987

II.C.i.c. Phospholipids

Summary

Phospholipids were measured by hydrolysis with phospholipase D to form choline and phosphatidic acids. The choline was oxidized by choline oxidase to betaine and hydrogen peroxide which was coupled to aminophenazone and phenol to form a dye complex which was monitored at 546 nm:

$$\text{phospholipids} + H_2O \xrightarrow{\text{phospholipase D}} \text{choline} + \text{phosphatidic acids}$$

$$\text{choline} + 2O_2 + H_2O \xrightarrow{\text{choline oxidase}} \text{betaine} + 2H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{phenol} \xrightarrow{\text{POD}} \text{4-(p-benzo-quinone-mono-imino)phenazone} + 4H_2O$$

Sensitivity:	0.2 mmol/L
Linearity:	0-12.9 mmol/L
Precision:	4%

Sample

Serum, heparinized plasma or EDTA plasma.

Reagents

BMC Phospholipids test kit, cat. no. 691844

1. Buffer: Tris hydroxymethyl aminomethane 50 mmol/L, pH 8.0, phenol 20 mmol/L.

2. Enzymes:

Phospholipase D	≥ 1000 U/L
Coline Oxidase	≥ 1400 U/L
Peroxidase	≥ 800 U/L
4-Aminophenazone	8 mmol/L

3. Standard: Choline Chloride 54.1 mg/100 mL = 3.88 mmol/L. Stable until expiry date on bottle at 2-8°C.

Quality Control

BMC Precinorm, Ciba-Corning ADS and Dade Monitrol 1 & 2 chemistry controls.

Reagent Preparation

The contents of bottle 2 were dissolved in 40 mL buffer from bottle 1. Stable 2 weeks at +2 -+8°C, 48 hrs at +15 - +25°C.

Procedure

1. Reagent was prepared as above.
2. 50 µL of QC and patient samples were pipetted into the appropriate sample cups and the sample rack was placed on the Cobas.
3. With power on, the appropriate test NR was entered (16).
4. Using step par, the assay parameters were checked.

Parameter Listing

1. Units	mmol/L
2. Calculation factor	0
3. Standard 1 conc.	1.94
4. Standard 2 conc.	3.88
5. Standard 3 conc.	3.88
6. Limit	12.9
7. Temperature (Deg. C)	37.0
8. Type of analysis	1
9. Wavelength (nm)	546
10. Sample volume (µL)	10
11. Diluent volume (µL)	20
12. Reagent volume (µL)	350
13. Incubation time (sec)	0
14. Start reagent volume (µL)	0
15. Time of first reading (sec)	.5
16. Time interval (sec)	60
17. Number of readings	04
18. Blanking mode	1
19. Printout mode	1

5. The contents of reconstituted bottle 2 were poured into the primary well of a reagent tray.

6. 50 µL of standard was diluted with 50 µL distilled water and placed in the first standard well of the tray. 100 µL of undiluted standard was pipetted in each of the 2nd and 3rd standard well.

7. The tray cover was attached and placed on Cobas.

8. The photometer arm was lifted and the cuvette ring was placed on the rotor. The clamping ring was positioned and the photometer arm was lowered.

9. The plexiglass cover was closed and start was pressed.

10. On completion, QC results were checked and logged. Over-limit results (flagged) were checked for. These were diluted with saline and re-run.

Interferences

Hemoglobin Bilirubin Ascorbic Acid	Do not interfere in usual concentration

Clinical Significance

Phospholipids are polar molecules found on the outer shell of lipoprotein particles. Normal values are usually 2.04-3.66 mmol/L. Phospholipid levels are used for research purposes to evaluate lipid metabolism and may be used to determine total serum lipids from the equation:

$$\text{Total Lipid} = 1.677\ (TC\text{-}FC) + FC + TG + PL\ (\text{in g/L})$$

References

1. Boehringer Mannheim GmbH. Test-combination phospholipids. Enzymatic colorimetric test, Cat. No. 691844, February, 1987.

II.C.i.d. Triglycerides in Serum

Summary/Automated Colorimetry

Triglycerides were determined by enzymatic hydrolysis followed by estimation of the liberated glycerol by colorimetry. A 3 μL patient sample was mixed with buffered lipase, the resulting glycerol was phophorylated and oxidized and the peroxide produced was coupled to a chromogen formation using 4-aminophenazone. The single reagent system measured color formation bichromatically at 505 and 700 nm:

$$\text{Triglycerides} + 3H_2O \xrightarrow{\text{LIPASE}} \text{Glycerol} + \text{Fatty Acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-}PO_4 + \text{ADP}$$

$$H_2O_2 + \text{4-Aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{POD}} \text{4-(p-Benzoquinone)phenazone} + 2H_2O + HCl$$

Linearity: 0-11.4 mmol/L
Sensitivity: 0.1 mmol/L
Precision: 1.8 per cent CV at 2.0 mmol/L

Sample

Serum: SST.

Interference:

Hemolysis - no effect from mild to moderate (+ to ++); severely hemolysed (+++) samples were not be run or reported.

Bilirubin - no effect up to 340 μmol/L.

Lipemia - no effect from light to moderate lipemia (up to 8.8 mmol/L triglyceride). Grossly lipemic samples could be cleared using the Beckman Airfuge (10 minutes at 30 psi).

Reagents

R1 - Reagent - BMC cat. no. 1201301/300 mL: tris buffer, 150 mmol/L, pH 7.6; $MgSO_4$, 17.5 mmol/L;
EDTA, 10 mmol/L;

p-chlorophenol, 3.5 mmol/L;
ATP, 0.5 mmol/L;
lipase, 3000 U/L;
4-aminophenazone, 0.35 mmol/l;
peroxidase, 150 U/L;
glycerol phosphate oxidase, 2500 U/L;
glycerol kinase, 200 U/L.

The contents of the GPO vial were dissolved in the 300 mL of buffer: the reagent was divided between the two analyzers. The working reagent is stable for two weeks when stored at 4°C.

Procedure

1. It was ensured that the proper system parameters were loaded into both Hitachi instruments using SYS 9, 17 ie:

(1) Assay code	ENDP-20
(2) Sample volume (µL)	3
(3) R1 volume (µL)	300
(4) R2 volume (µL)	0
(5) Wavelength 1 (nm)	505
Wavelength 2 (nm)	700
(7) Calibration 01 Saline	0
02 Cal	See defined value
(8) Equation	1
(9) Unit factor	See defined value

2. If fresh reagent was added, the volume was adjusted using RTN-1 a full prime using PRIMING DISC in RTN-1 was done, followed by MTN-2.

3. Calibrators using RTN-2 and QC's and controls using RTN-5 were requested.

4. A Q listing was prepared in the Cerner LIS system under WQM, work-center 110, site 110, matching the 3 digit stamped sequence number against the 7 digit requisition number.

5. A minimum of 200 µL of patient sample was transferred to the pre-numbered sample cup and barcode reader tube bearing the same stamped sequence number as the patient specimen.

6. The patient sample cup/barcode tube was loaded into any open position on a sampling carousel, with appropriate QC's (2) and controls (2) in positions 1, 2, 20 and 40 as required.

7. The carousel was loaded into one of the Hitachi analyzers, and RUN was typed in from the home position of any screen indicating tray and cup number (RUN X XX ENTER). The barcode reader was monitored from RTN-3.

8. After the patient results were printed, a copy of the patient requisition was stapled to the report; patient ID, sequence number and test selection were verified. The patient results were checked for codes, linearity limits or non-conforming results.

9. Samples above the linearity limit were diluted 1:1 or 1:2 with saline and re-run; the analyzer results were multiplied by 2 or 3.

10. The report was initialled before sending for computer entry.

Reference Range

Serum/plasma: 0.55-1.7 mmol/L (adult fasting)

Clinical Significance

Increased Serum Triglycerides	Decreased Serum Triglycerides
Hyperlipoproteinemias	Chronic obstructive lung disease
Alcoholic cirrhosis	Malnutrition
Biliary cirrhosis	Malabsorption syndrome
Pancreatitis	Hyperthyroidism
Diabetes mellitus	

References

1. BMC Hitachi 737 Operating Reference Manual

2. Tietz N.W., Fundamentals of Clinical Chemistry, pp 474-81, 1987.

II.C.ii. Clinical Chemistry

II.C.ii.a. Alanine Aminotransferase in Serum

Summary/Automated Rate Colorimetry

Alanine aminotransferase (ALT) was determined colorimetrically based on the alanine to pyruvate conversion, coupled with UV rate measurement of NAD formation. A 10 µL patient sample was mixed with buffer, alanine, NADH, lactic dehydrogenase and keto-glutarate. The decrease in NADH was measured bichromatically at 340 and 415 nm, and the rate was proportional to the activity/concentration of the alanine aminotransferase.

$$\alpha\text{-Ketoglutarate} + \text{L-Alanine} \xrightarrow{\text{ALT}} \text{L-Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} \xrightarrow{\text{LD}} \text{Lactate} + \text{NAD}$$

Linearity: 0-800 U/L
Sensitivity: 1 U/L
Precision: 4.2 per cent CV at 24 U/L

Sample

Serum: SST.

Interference:

Hemolysis - minimal effect from mild (+); hemolysis; moderate or severely hemolysed (++ to +++) samples were not run or reported.

Bilirubin - no effect up to 340 µmol/L.

Lipemia - no effect from light to moderate lipemia (up to 8.8 mmol/L triglyceride). Grossly lipemic samples could be cleared using the Beckman Airfuge (10 minutes at 30 psi).

Reagents

R1 - Buffer - BMC cat. no. 1201280/200 mL: phosphate buffer, 104 mmol/L pH 7.4:
L-alanine, 1040 mmol/L;
NADH, 0.23 mmol/L;
LD, 1.5 U/L.

The contents of 1a bottle (8 tablets) were dissolved in the 200 mL of buffer. The reconstituted reagent was stable 7 days when stored capped at 4°C.

R2 - Ketoglutarate - BMC cat. no. 1127845/300 mL: the reagent as supplied was stable until the specified expiry date when stored at 4°C.

Procedure

1. It was ensured that the proper system parameters were loaded into both Hitachi instruments using SYS 9, 9 ie:

(1)	Assay code	RATE-11-20
(2)	Sample volume (μL)	10
(3)	R1 volume (μL)	250
(4)	R2 volume (μL)	50
(5)	Wavelength 1 (nm)	340
	Wavelength 2 (nm)	415
(7)	Calibration 01 Saline	0
	02 Cal	See defined value
(8)	Equation	1
(9)	Unit factor	See defined value

2. If fresh reagent was added, the volume was adjusted using RTN-1 and a full prime was done using PRIMING DISC in RTN-1, followed by MTN-2.

3. Calibrators using RTN-2 and QC's and controls using RTN-5 were requested.

4. A Q listing was prepared in the Cerner LIS system under WQM, work-center 110, site 110, matching the 3 digit stamped sequence number against the 7 digit requisition number.

5. A minimum of 200 μL of patient sample was transferred to the pre-numbered sample cup and barcode reader tube bearing the same stamped sequence number as the patient specimen.

6. The patient sample cup/barcode tube was loaded into any open position on a sampling carousel, with appropriate QC's (2) and controls (2) in positions 1, 2, 20 and 40 as required.

7. The carousel was loaded into one of the Hitachi analyzers, and RUN was typed in from the home position of any screen indicating tray and cup number (RUN X XX ENTER). The barcode reader was monitored from RTN-3.

8. After the patient results were printed, a copy of the patient requisition was stapled to the report; The patient ID, sequence number and test selection were verified. The patient results were checked for codes, linearity limits or non-conforming results.

9. Samples above the linearity limit were diluted 1:2 or 1:4 with saline and re-run; The analyzer results were multiplied by 3 or 5.

10. The report was initialled before sending for computer entry.

Reference Range

Serum/plasma: 5-40 U/L (adult)

Clinical Significance

Increased Serum Alanine Aminotransferase

Liver cell necrosis	Skeletal muscle trauma
Myositis	Myocarditis
Cirrhosis	Obstructive jaundice
Myocardial infarction	Hemolytic diseases

References

1. BMC Hitachi 737 Operating Reference Manual

2. Tietz NW, Fundamentals of Clinical Chemistry, 1987, pp 369-73

II.C.ii.b. Albumin in Serum

Summary/Automated Colorimetry

Albumin was determined colorimetrically using bromcresol green dye binding. A 3 µL patient sample was mixed with the bromcresol green reagent which was buffered at a pH of 4.2. The albumin-BCG complex was measured (end-point) at 600 and 700 nm and converted to an albumin concentration in g/L.

Albumin + Bromcresol Green (BCG) ----- ► BCG-Albumin Complex

Linearity:	0-50 g/L
Sensitivity:	1 g/L
Precision:	1.5 per cent CV at 41 g/L

Sample

Serum: SST.

Interference:

Hemolysis - no effect from mild to moderate (+ to ++); severely hemolysed (+++) samples were not run or reported.

Bilirubin - no effect up to 340 µmol/L.

Lipemia - no effect from light to moderate lipemia (up to 8.8 mmol/L triglyceride). Grossly lipemic samples could be cleared using the Beckman Airfuge (10 minutes at 30 psi).

Reagents

R1 - Buffer/BCG - BMC cat. no. 1127446/600 mL: succinate buffer, 75 mmol/L ph 4.2, bromcresol green, 0.15 mmol/L. Reagent was stable until specified expiry date when stored at room temperature.

Procedure

1. It was ensured that the proper system parameters had been loaded into both Hitachi instruments using SYS 9, 6 ie:

(1)	Assay code	ENDP-02
(2)	Sample volume (µL)	3
(3)	R1 volume (µL)	350
(4)	R2 volume (µL)	0
(5)	Wavelength 1 (nm)	600
	Wavelength 2 (nm)	700
(7)	Calibration 01 Saline	0
	02 Cal	See defined value
(8)	Equation	1
(9)	Unit factor	See defined value

2. If fresh reagent was added, the volume was adjusted using RTN-1 and a full prime using PRIMING DISC in RTN-1 was done, followed by MTN-2.

3. Calibrators using RTN-2 and QC's and controls using RTN-5 were requested.

4. A Q listing was prepared in the Cerner LIS system under WQM, work-center 110, site 110, matching the 3 digit stamped sequence number against the 7 digit requisition number.

5. A minimum of 200 µL of patient sample was transferred to the pre-numbered sample cup and barcode reader tube bearing the same stamped sequence number as the patient specimen.

6. The patient sample cup/barcode tube was loaded into any open position on a sampling carousel, with appropriate QC's (2) and controls (2) in positions 1, 2, 20 and 40 as required.

7. The carousel was loaded into one of the Hitachi analyzers, and RUN was typed in from the home position of any screen indicating tray and cup number (RUN X XX ENTER). The barcode reader was monitored from RTN-3.

8. After the patient results were printed, a copy of the patient requisition was stapled to the report; the patient ID, sequence number and test selection were verified. The patient results were checked for codes, linearity limits or non-conforming results.

9. Samples above the linearity limit were diluted 1:1 with saline and re-run; the analyzer results were multiplied by 2.

10. The report was initialled before sending for computer entry.

Reference Range

Serum/plasma: 35-48 gl/L (adult)

Clinical Significance

Decreased Serum Albumin	Increased Serum Albumin
Protein malnutrition	Dehydration
Thermal burns	Albumin hyperinfusion
Nephrotic syndrome	
Intestinal malabsorption	
Chronic inflammatory bowel disease	
Severe diffuse liver disease	
Excessive fluid treatment	
Severe thyrotoxicosis	

References

1. BMC Hitachi 737 Operating Reference Manual

2. Tietz NW, Fundamentals of Clinical Chemistry, 1987, pp 328-30

II.C.ii.c. Alkaline Phosphatase in Serum

Summary/Automated Rate Colorimetry

Alkaline phosphatase (ALP) was determined colorimetrically using p-nitrophenyl phosphate. A 10 µL patient sample was mixed with buffer and p-nitrophenyl phosphate and the formation of p-nitrophenol was measured bichromatically at 415 and 660 nm; the rate of formation being proportional to the activity/concentration of the alkaline phosphatase.

$$\text{p-Nitrophenyl Phosphate} + H_2O \xrightarrow{\text{ALP}} \text{p-Nitrophenol} + PO_4$$

Linearity:	0-1200 U/L
Sensitivity:	1 U/L
Precision:	1.7 per cent CV at 75 U/L

Sample

Serum: SST.

Interference:

Hemolysis - minimal effect from mild (+); hemolysis; moderate to severely hemolysed (++ to +++) samples were not run or reported.

Bilirubin - no effect up to 340 µmol/L.

Lipemia - no effect from light to moderate lipemia (up to 8.8 mmol/L triglyceride). Grossly lipemic samples could be cleared using the Beckman Airfuge (10 minutes at 30 psi).

Reagents

R1 - Buffer - BMC cat. no. 1358278/300 mL: 2-amino-2-methyl-1-propanol buffer, 0.36 mol/L, pH 10.4;
Mg++, 2.84 mmol/L;
Zn++, 1/43 mmol/L;
EDTA, 1.43 mmol/L.

The contents of 1a bottle were dissolved in the 300 mL of buffer. The reagent was divided between the two analyzers. The reconstituted reagent was stable 3 days when stored capped at 4°C.

R2 - Substrate - BMC cat. no. 1358286/100 mL: p-nitrophenyl phosphate, 57.1 mmol/L; 2-amino-2-methyl-1-propanol buffer, 0.36 mol/L, pH 10.4.

The contents of 2a bottle were dissolved in the 100 mL of buffer. The reconstituted reagent was stable days when stored capped at 4°C.

Procedure

1. It was ensured that the proper system parameters were loaded into both Hitachi instruments using SYS 9, 13 ie:

(1) Assay code	RATE-11-20
(2) Sample volume (µL)	73
(3) R1 volume (µL)	250
(4) R2 volume (µL)	100
(5) Wavelength 1 (nm)	415
Wavelength 2 (nm)	660
(7) Calibration 01 Saline	0
02 Cal	See defined value
(8) Equation	1
(9) Unit factor	See defined value

2. If fresh reagent was added, the volume was adjusted using RTN-1 and a full prime was done using PRIMING DISC in RTN-1, followed by MTN-2.

3. Calibrators using RTN-2 and QC's and controls using RTN-5 were requested.

4. A Q listing was prepared in the Cerner LIS system under WQM, work-center 110, site 110, matching the 3 digit stamped sequence number against the 7 digit requisition number.

5. A minimum of 200 µL of patient sample was transferred to the pre-numbered sample cup and barcode reader tube bearing the same stamped sequence number as the patient specimen.

6. The patient sample cup/barcode tube was loaded into any open position on a sampling carousel, with appropriate QC's (2) and controls (2) in positions 1, 2, 20 and 40 as required.

7. The carousel was loaded into one of the Hitachi analyzers, and RUN was typed in from the home position of any screen indicating tray and cup number (RUN X XX ENTER). The barcode reader was monitored from RTN-3.

8. After the patient results were printed, a copy of the patient requisition was stapled to the report; Patient ID, sequence number and test selection were verified. The patient results were checked for codes, linearity limits or non-conforming results.

9. Samples above the linearity limit were dilute 1:2 or 1:4 with saline and re-run; the analyzer results were multiplied by 3 or 5.

10. The report was initialled before sending for computer entry.

Reference Range

Serum/plasma:
20 - 55 yrs - 36 - 135 U/L
55 - 74 yrs - 37 - 160 U/L
> 75 yrs - 50 - 200 U/L

Clinical Significance

Increased Serum Alkaline Phosphatase

Increased bone metabolism
Bone metastases
Osteogenic sarcoma
Multiple myeloma
Infectious mononucleosis

Extrahepatic biliary obstruction
Cholangitis
Hepatocellular jaundice
Cirrhosis

References

1. BMC Hitachi 737 Operating Reference Manual

2. Tietz NW, Fundamentals of Clinical Chemistry, 1987, pp 385-91

II.C.ii.d. Aspartate Aminotransferase in Serum

Summary/Automated Rate Colorimetry

Aspartate aminotransferase (AST) was determined colorimetrically based on the aspartate to oxaloacetate conversion, coupled with UV rate measurement of NAD formation. A 10 µL patient sample was mixed with buffer, aspartate, NADH, malate dehydrogenase and ketoglutarate. The rate of decrease in the NADH was measured bichromatically at 340 and 415 nm, and was proportional to the activity/concentration of the aspartate aminotransferase.

$$\alpha\text{-Ketoglutarate} + \text{L-Aspartate} \xrightarrow{\text{AST}} \text{L-Glutamate} + \text{Oxaloacetate}$$

$$\text{Oxaloacetate} + \text{NADH} \xrightarrow{\text{MDH}} \text{Malate} + \text{NAD}$$

Linearity: 0-1000 U/L
Sensitivity: 1 U/L
Precision: 2.8 per cent CV at 33 U/L

Sample

Serum: SST.

Interference:

Hemolysis - minimal effect from mild (+); hemolysis; moderate or severely hemolysed (++ to +++) samples were not run or reported.

Bilirubin - no effect up to 340 µmol/L.

Lipemia - no effect from light to moderate lipemia (up to 8.8 mmol/L triglyceride). Grossly lipemic samples could be cleared using the Beckman Airfuge (10 minutes at 30 psi).

Reagents

R1 - Buffer - BMC cat. no. 1201271/200 mL: phosphate buffer, 104 mmol/L pH 7.4;
L-aspartate, 260 mmol/L;
NADH, 0.23 mmol/L;
MD, 0.7 U/L;
LD, 1.2 U/L.

The contents of 1a bottle (8 tablets) were dissolved in the 200 mL of buffer. The reconstituted reagent was stable 7 days when stored capped at 4°C.

R2 - Ketoglutarate - BMC cat. no. 1127829/300 mL: the reagent as supplied was stable until the specified expiry date when stored at 4°C.

Procedure

1. It was ensured that the proper system parameters were loaded into both Hitachi instruments using SYS 9, 10 ie:

(1) Assay code	RATE-11-20
(2) Sample volume (µL)	10
(3) R1 volume (µL)	250
(4) R2 volume (µL)	50
(5) Wavelength 1 (nm)	340
Wavelength 2 (nm)	415
(7) Calibration 01 Saline	0
02 Cal	See defined value
(8) Equation	1
(9) Unit factor	See defined value

2. If fresh reagent was added, the volume was adjusted using RTN-1 and a full prime was done using PRIMING DISC in RTN-1, followed by MTN-2.

3. Calibrators using RTN-2 and QC's and controls using RTN-5 were requested.

4. A Q listing was prepared in the Cerner LIS system under WQM, work-center 110, site 110, matching the 3 digit stamped sequence number against the 7 digit requisition number.

5. A minimum of 200 µL of patient sample was transfered to the pre-numbered sample cup and barcode reader tube bearing the same stamped sequence number as the patient specimen.

6. The patient sample cup/barcode tube was loaded into any open position on a sampling carousel, with appropriate QC's (2) and controls (2) in positions 1, 2, 20 and 40 as

required.

7. The carousel was loaded into one of the Hitachi analyzers, and RUN was typed in from the home position of any screen indicating tray and cup number (RUN X XX ENTER). The barcode reader was monitored from RTN-3.

8. After the patient results were printed, a copy of the patient requisition wqs stapled to the report; The patient ID, sequence number and test selection were verified. The patient results were checked for codes, linearity limits or non-conforming results.

9. Samples above the linearity limit were diluted 1:2 or 1:4 with saline and re-run; the analyzer result was multiplied by 3 or 5.

10. The report was initialled before sending for computer entry.

Reference Range

Serum/plasma: 10-30 U/L (adult)

Clinical Significance

Increased Serum Aspartate Aminotransferase

Liver cell necrosis
Hepatitis
Obstructive jaundice
Pulmonary embolism
Skeletal muscle trauma
Cirrhosis
Myocardial infarction

References

1. BMC Hitachi 737 Operating Reference Manual
2. Tietz NW, Fundamentals of Clinical Chemistry, 1987, pp 369-73

II.C.ii.e. Gamma Glutamyl Transferase in Serum

Summary/Automated Rate Colorimetry

Gamma glutamyl transferase (GGT) was determined colorimetrically using the substrate γ-glutamyl-3-carboxy-4-nitroanalide. An 8 μL patient sample was mixed with buffer and glycylglycine to which the substrate was added and the rate of 5-amino-2-nitrobenzoate formation was measured bichromatically at 415 and 660 nm, the rate being proportional to the activity/concentration of γ-glutamyl transferase.

$$\gamma\text{-Glutamyl-3-carboxy-4-nitroanalide} + \text{Glycylglycine} \xrightarrow{\text{GGT}}$$

$$\gamma\text{-Glutamyl-glycylglycine} + \text{5-amino-2-nitrobenzoate}$$

Linearity:	0-1200 U/L
Sensitivity:	1 U/L
Precision:	5.6 per cent CV at 21 U/L

Sample

Serum: SST.

Interference:

Hemolysis - minimal effect from mild (+) hemolysis; moderate or severely hemolysed (++ to +++) samples were not run or reported.

Bilirubin - no effect up to 340 μmol/L.

Lipemia - no effect from light to moderate lipemia (up to 8.8 mmol/L triglyceride). Grossly lipemic samples could be cleared using the Beckman Airfuge (10 minutes at 30 psi).

Reagents

R1 - Buffer - BMC cat. no. 1360809/400 ml: tris buffer, 143 mmol/L, pH 8.25; glycylglycine, 143 mmol/L.

The contents of the bottle la (granulate) were dissolved in 400 mL of buffer reagent.

The reconstituted reagent was stable for 3 months when stored capped at 4°C.

R2 - Substrate Reagent - BMC cat. no. 1355058/200 mL: γ-glutamyl-3-carboxy-4-nitroanalide, 10.4 mmol/L.

The contents of bottle 2a (granulate) were dissolved in the 200 mL of diluent. The reconstituted reagent was stable for 3 weeks when stored capped at 4°C.

Procedure

1. It was ensured that the proper system parameters had been loaded into both Hitachi instruments using SYS 9, 16 ie:

(1)	Assay code	RATE-11-20
(2)	Sample volume (μL)	8
(3)	R1 volume (μL)	300
(4)	R2 volume (μL)	60
(5)	Wavelength 1 (nm)	415
	Wavelength 2 (nm)	660
(7)	Calibration 01 Saline	0
	02 Cal	See defined value
(8)	Equation	1
(9)	Unit factor	See defined value

2. If fresh reagent was added, the volume was adjusted using RTN-1 and a full prime using PRIMING DISC in RTN-1 was done, followed by MTN-2.

3. The calibrators using RTN-2 and QC's and controls using RTN-5 were requested.

4. A Q listing was prepared in the Cerner LIS system under WQM, work-center 110, site 110, matching the 3 digit stamped sequence number against the 7 digit requisition number.

5. A minimum of 200 μL of patient sample was transferred to the pre-numbered sample cup and barcode reader tube bearing the same stamped sequence number as the patient specimen.

6. The patient sample cup/barcode tube was loaded into any open position on a sampling carousel, with appropriate QC's (2) and controls (2) in positions 1, 2, 20 and 40 as required.

7. The carousel was loaded into one of the Hitachi analyzers, and RUN was typed in from the home position of any screen indicating tray and cup number (RUN X XX ENTER). The barcode reader was monitored from RTN-3.

8. After the patient results were printed, a copy of the patient requisition was stapled to the report; The patient ID, sequence number and test selection were verified. The patient results were checked for codes, linearity limits or non-conforming results.

9. Samples above the linearity limit were diluted 1:2 or 1:4 with saline and re-run; the analyzer results were multiplied by 3 or 5.

10. The report was initialled before sending for computer entry.

Reference Range

Serum/plasma: 8-50 U/L (male); 6-50 U/L (female)

Clinical Significance

Increased Serum γ-Glutamyl Transferase

Obstructive liver disease	Cirrhosis
Post hepatic obstruction	Chronic alcoholism
Infectious mononucleosis	

References

1. BMC Hitachi 737 Operating Reference Manual

2. Tietz NW, Fundamentals of Clinical Chemistry, 1987, pp 391-2

II.C.iii. Thyroid Hormones and Thyroid Function

II.C.iii.a. Free T4

Summary

The Amerlex-MAB FT4 kit is a direct, competitive assay for free thyroxine. Tracer antibody is mixed with the sample, establishing equilibrium. The proportion of I^{125} monoclonal anti-T4 tracer antibody bound to the separation suspension particles is inversely related to the concentration of the FT4 present in the sample. Separation of the particle-bound tracer is accomplished by placing the tubes in the magnetic racks and the supernatant is decanted. Reference standards are set up simultaneously with the patient sera and are used to determine the concentrations of the unknown sera.

Sensitivity:	0.6 pmol/L
Linearity:	0-130 pmol/L
Precision:	6-12%

Sample

Serum and plasma (heparin & EDTA).
SST or red-stoppered tube spun with Sure-Sep.
Unacceptable samples: repeated thawing, gross lipemia, gross hemolysis, very icteric.

Sample preparation and storage: separate serum in plastic tubes and stored in sample fridge.

Sample stability: 2.8°C for up to 7 days; longer at -20°C.

Reagents

Amersham 400 test pack, code 1 M 5054. All reagents were supplied ready to use.

1. Two sets of 6 vials free T4 standards in liquid human serum (1 mL each);
   A - 0 pmol/L;
   B - 2.5 pmol/L;
   C - 10.0 pmol/L;
   D - 25.0 pmol/L;
   E - 60.0 pmol/L;
   F - 130.0 pmol/L.

2. Four bottles Amerlex-MAB separation suspension (magnetisable polymer-ligand

particle suspension), 55 mL each, coloured blue-black with anti-microbial agent.

3. Four bottles I^{125} monoclonal anti-T4 tracer antibody solution, 55 mL each, coloured red with anti-microbial agent.

4. Package insert.

Procedure

All tubes were treated identically. Reagents were stored at 4°C and were brought to room temperature. Reagents and specimens were mixed thoroughly before use.

Excess foaming of reagents was avoided.

The same lot number was used for all reagents and they were not used past the expiry date.

A standard curve and QCs were included with each run.

1. Tubes were numbered in duplicate as follows:

Tube	Std.	QC	Unknown
1,2	0 - A		
3,4	2.5 - B		
5,6	10.0 - C		
7,8	25.0 - D		
9,10	60.0 - E		
11,12	130.0 - F		
13,14	TC		
15,16		QC1	
17,18		QC2	
19,20		QC3	
21,22			1
etc.			etc.

2. Using an SMI pipette, 50 µL of standard, QC and pts were added to appropriately labelled tubes.

3. Using an Eppendorf Repeater pipette, 500 µL of separation suspension reagent was dispensed to each tube except TC (13,14).

4. With the repeater pipette, 500 µL of I^{125} tracer was dispensed to all tubes including TC (13,14). Tubes 13 and 14 were set aside.

5. All tubes were vortexed thoroughly. Tubes were covered and incubated 30 minutes in a 37°C water bath.

6. All tubes were placed in magnetic racks for 15 minutes.

7. Supernatant was poured off, tubes were blotted and allowed to drain for 5 minutes.

8. Leaving tubes inverted, any remaining liquid was aspirated off.

9. All tubes were counted, including TC in gamma counter program AO4.

   Reportable units: < 2 - > 130 pmol/L.

Reference Range

11.0 - 23.0 pmol/L (April 8, 1992)

Clinical Significance

Abnormal T4 levels may arise from a variety of causes apart from thyroid malfunction. Since the function of the thyroid is mediated by the level of TSH which is derived from the pituitary, any malfunction of the latter system will also influence the degree of thyroid stimulation. For euthyroid individuals, a change in TBG concentration will result in a parallel alteration of the circulating free T4. A rise in TBG levels associated with pregnancy or estrogens such as oral contraceptives, elevates serum T4. Androgen or anabolic steroids lower T4 levels by decreasing TBG concentration. Any factor that influences the extent of T4 binding to the existing level of protein will also influence T4 levels. Drugs such as phenytoin and salicylates lower T4 levels by interfering with T4 binding to TBG, and in the case of salicylates, binding to the thyroxine-binding prealbumin (TBPA) and albumin (TA) as well as TBG.

The free T4 fraction of thyroxine is the physiologically active fraction. Primary malfunction of the thyroid gland may lead to excessive or subnormal release of free T4. Malfunction of the hypothalamic-pituitary axis influences the degree of stimulation of the thyroid gland and its activity. Measurement of free T4 is used to assess thyroid status.

References

1. Amersham. Amerlex-MAB FT4 package insert, 1991. (20 references included)

II.C.iii.b. Thyroid Stimulating Hormone (TSH)

Thyroid Stimulating Hormone (TSH) or thyrotrophin, is a glycoprotein with a molecular weight of 28,000 secreted by the thyroid gland. The TSH molecule has 2 sub units, α and β. The unit is similar to units of other pituitary hormones, e.g. L.H., FSH but the β sub unit gives biological specificity to the whole molecule which can therefore be distinguished in immunoassays.

The target organ of TSH is the thyroid gland which is responsible for releasing thyroxine (T_4) and Triiodothyronine (T_3). These hormone levels exert a negative feedback on the pituitary, inhibiting the release of TSH, and thereby preserving a metabolic equilibrium.

Abnormalities in this cycle can result in overproduction (hyperthyroidism) or underproduction (hypothyroidism).

Principle and Purpose of Test

The kit used for the measurement of TSH was the TSH MAIA clone. This technology incorporates two high affinity monoclonal antibodies into an immunoradiometric assay. Standards, quality control and specimens react with a mixture of monoclonal antibodies to TSH. The ^{125}I labelled antibody attaches to a unique site on the TSH molecule, and a second monoclonal antibody linked to fluorescein binds at a discrete site on the TSH molecule to form a sandwich.

At the end of a one hour incubation, antifluorescein coupled to a magnetic solid phase is added in excess, binds specifically to the TSH monoclonal antibody complex, and is separated in a magnetic field. A wash step reduces non-specific binding to enhance low end precision.

The concentration of antigen is directly proportional to the radioactivity bound to the separating agent.

Specimen Requirements

A serum specimen was used in this analysis.

a) A 9.5 mL venous specimen was collected in a Serum Separator Tube (SST, BD #6510). The blood was allowed to clot at room temperature after which the tube was centrifuged and the serum fraction separated.

Specimens could be stored at 2-8°C for 24 hours, or at -20°C for up to 30 days.

Repeated thawing and freezing was avoided. Specimens were thoroughly mixed before assay.

Reagents

(1) **^{125}I Anti TSH Reagent**

6.6 mL per vial containing fluorescein labelled and ^{125}I labelled mouse monoclonal antibodies to TSH. < 270 k Bq < 10 µCi per vial [red colour].

(2) **Standards**

7 vials of standards

a)	0	µIV/ml
b)	0.15	
c)	0.5	
d)	1.5	
e)	5	
f)	15	
g)	50	

Standards were calibrated against (2nd IRP 80/558) in bovine serum with sodium azide 0.2% (w/v).

(3) **TSH MAIA Clone Separating Reagent**

26.4 mL per vial containing sheep anti serum to fluorescein, bound to magnetic particles.

(4) **TSH MAIA Clone Wash Buffer Concentrate**

12.5 mL per vial containing TRIS buffer. Contents were diluted to 100 mL with distilled water.

All reagents were stored at 2-8°C until expiry date on label.

Quality Control

i) **Serotest: Lyophilized control containing human serum with Thimerosal 0.1% (w/v).**

Reconstituted with 1 mL distilled H_2O and left standing ½ hour. Several vials which were pooled were mixed gently, then aliquoted, frozen and stored at -20°C.

ii) **LYPHOCHEK Immunoassay Control Serum, levels I, II & III.**

Lyphophilized human serum. Reconstituted with 5 mL distilled water, allowed to stand 15 mins. Aliquoted and stored at -20°C.

iii) In house pooled sera: Previously run samples with ranges of 4-6 µIV/L, 7-10 µIV/L 12-15 µIV/L, 18-21 µIV/L and 20-2 µIV/L were pooled, aliquoted and frozen. 20-30 values were obtained on each pool and a value was calculated.

- **External Controls:** Murex Immunoassay Quality programme 1 was used. 12 vials of lyophilized controls were run over a period of six months. LPTP controls were also run.

Equipment/Calibration

Required for assay:

12 x 75 mm round bottom plastic tubes
0.2 mL SMI micro pipet
Eppendorf repettor
100 mL Volumetric flask
Vortex
MAIA Magnetic Separator and rack (Cat. No. 00992)
Gamma scintillation counter calibrated to detect ^{125}1
Pipet (SMI) calibration checked weekly by inserting 0.2 mL calibration rod in pipet fitted with glass tip. Edge of tip and rod should be flush.
Background count of γ counter checked weekly by placing empty counting rack in counter and counting for 1 minute. Background count of less than 35 was acceptable.

Assay Procedure for TSH

1. Specimens and reagents were brought to room temperature. Specimens were mixed thoroughly. Reagents were mixed gently.

2. 12 x 75 mm polypropylene tubes were labelled.

3. 200 µL aliquots of standards, quality control and specimen were pipetted in duplicate in the corresponding tube.

4. With Eppendorf dispenser, 0.1 mL of ^{125}I TSH reagent was added to each tube. The entire rack was shaken using side to side motion.

5. Incubated 1 hour at room temperature.

6. The wash solution was diluted to 100 mL.

7. The separation reagent was placed on the rotator about 20 minutes before the end of incubation.

8. Using Eppendorf repeating dispenser, 0.2 mL of well mixed separation reagent was added to each tube. Reagent was swirled after each row. The entire rack was shaken using side to side motion and incubated 5 minutes at room temperature.

9. The rack of tubes was slid into the MAIA magnetic separator and let to stand 2 minutes. It was ensured that the bottom of all tubes touched the surface of the separator.

10. The supernatant was decanted by inverting the magnetic separator. The inverted separator was placed on the absorbent pad and the base was tapped firmly to dislodge excess droplets.

11. The separator was returned to upright position and 0.5 mL diluted wash solution was added.

12. The tubes were well vortexed and let to stand 2 minutes on the separator. It was ensured that all tubes touched the surface of the separator.

13. Decanted by smooth inversion of magnetic separator. Inverted separator was placed on absorbent paper. The base of separator was firmly tapped to dislodge any droplets adhering to side of tubes and let to drain 5 minutes.

14. Counted on LKB γ counter for 1 minute under program 10.

TSH MAIACLONE ASSAY

Procedure

Reagents were allowed to warm up to room temperature. 12 x 75 mm plastic tubes were labelled for standards, quality control and unknown.

Pipetted as follows:

Tube No.	1 2	3 4	5 6	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24	25 26
0.2 mL	TC (none)	A std 0 REFR	B std .15	C std .5	D std 1.5	E std 5	F std 15	G std 50	QC	QC	QC	UNKS	UNKS
Add	.1 mL 1-125 Anti-TSH Reagent												
	Shake rack. Incubate 1 hr. at room temperature.												
Add (except tubes 1 and 2)	0.2 mL TSH-IRMA Separation Reagent.												
	Shake rack. Incubate 5 minutes at room temperature.												
	Place on magnetic separator for 2 minutes/Decant and blot tubes.												
Add (except tubes 1 and 2)	0.5 mL wash buffer. Vortex. Place on magnetic separator for 2 minutes.												
	Decant. Drain for 5 minutes. Blot tubes.												
	Count on γ counter Prog. 10.												
	Normal range: 0.43 - 3.8 μIU/L (euthyroid). * Lowest level 0.1. High Limit greater than 50..												

If exact value was requested, dilution of serum with zero standard was made.

Reference Range

0.43 - 3.8 μIU/L (euthyroid)

References

1. Serono Diagnostics. TSH MAIAclone kit insert, pp. 2-13, 1992.

2. Pierce, J.C. Pituitary Thyrotcopin: Chemistry. In *The Thyroid.* S. Werner and S.H. Ingbar. Ed., Harper & Row, Hagerstown, MD, 1978.

3. Field, J.B. Pituitary Thyrotropin: mechanism of action. In *The Thyroid.* S. Werner and S.H. Ingbar, Ed., Harper & row, Hagerstown, MD, 1978.

4. Scanlon, M.F. and Smith, B.R. et al. Thyroid-stimulating hormone: neuro-regulation and clinical applications. *Clin. Sci. Mol. Med.* **55**, 1 and 129 (1978).

5. Reichlin, S. Neuroendocrine control. In *The Thyroid*, S. Werner and S.H. Ingbar, Ed., Harper & Row, Hagerstown, MD, 1978.

6. Eastham, R.D. Biochemical Values in Clinical Medicine, 7th Edition (1985). J. Wright and Sons Ltd., Bristol.

7. Rattle, S.J., Purnell, D.R., Williams, P., Siddle, K. and Forrest, G.C. (1984). New separation method for monoclonal immunoradiometric assays and its application to assays for thyrotropin and human choriogonadotropin. *Clin. Chem.*, **30**, 1457-1462.

II.C.iii.c. Total T_3

The principal thyroid hormones are triiodothyronine (T_3) and thyroxine (T_4). These hormones are synthesized by the thyroid gland and circulate in the blood bound to the proteins thyroxine binding globulin (TBG), pre albumin and albumin. The total serum concentration of T_3 is about 1/70 that of T_4 and is known to have more metabolic activity than T_4. Although T_3 is produced by the thyroid, a substantial part of the total serum T_3 seems to be derived from extra-thyroid deiodination of T_4.

Principle and Purpose of Test

The kit used for the measurement of total T_3 was the Amerlex - M T_3 RIA method. This Radio Immuno Assay method depends on the competition between triiodothyronine in the serum competing with ^{125}I triiodothyronine for a limited number of binding sites on a T_3 specific antibody when incubated at 37°C. The antibody suspension is bound to magnetic particles and separation of bound and free portions is carried out by sitting the mixture on a magnetic plate and then is carried out by sitting the mixture on a magnetic plate and then decanting the supernatant. The amount of bound T_3 to the antibody is inversely proportional to the concentration of T_3 present, so by running a series of standards the values of unknown samples can be interpolated.

Specimen Requirements

Serum specimen was required.

9.5 mL of venous specimen was collected in a Serum Separator Tube (SST, BD #6510) and allowed to clot at room temperature. The tube was then centrifuged and the serum fraction was separated.

Specimens could be stored at 2 - 8°C for 24 hours or at -20°C for up to 30 days.

Repeated thawing and freezing was avoided. Specimens were thoroughly mixed before assay.

Reagents

Amerlex - M T_3 RIA kit code IM 3001.

1. 1 bottle (55 mL, < 370 kBq/10 µCi ^{125}I triiodothyronine solution (red colour)).
2. 1 bottle (55 mL) Amerlex - M T_3 antibody suspension.
3. 6 vials T_3 standards in human serum.

Standards had lot specific values of ~ 0 - 12 nmol/L

4. 1 vial 5 mL Amerlex - M NSB reagent [Blue Green]. All reagents had a listed expiry date. Storage of all reagents was 2 - 8°C.

Quality Control

The quality controls used in this assay were the Lypochek Immunoassay Control Serum Levels I, II and III.

Lyophilized human serum. Reconstituted with 5 mL distilled water, allowed to stand 15 minutes. Aliquoted and stored at -20°C.

Pooled Sera

Previously assayed samples with low, normal and high values were pooled, aliquoted and frozen. ~ 20-30 values of each pool were obtained and a value was obtained.

Equipment/Calibration

Required for Assay:

12 x 75 round bottom plastic tubes
0.5 mL SMI micro pipet (0.1 mL with interchangeable head)
Eppendorf repettor
Vortex
Amerlex - M Separators (N 4001)
Water Bath - Metabolic Shaker Bath/Precision Scientific Co.
Gamma scintillation counter calibrated to detect ^{125}I

Assay temperature was 37 ± 1°C. The water level was approximately 1½ inches high. The temperature was checked before assay.

Pipet (SMI 0.1 mL) calibration rod in pipet fitted with glass tip. Edge of tip and rod should be flush.

Background count of γ counter was checked weekly by placing empty counting rack in counter and counting for 1 minute. Background count of less than 35 was acceptable.

Assay Protocol for Total T_3

1. It was checked that water bath temperature was 37°C.
2. 12 x 75 polypropylene tubes were labelled.
3. Specimens and reagents were brought to room temperature. Specimen was mixed thoroughly. Foaming of agents was avoided when mixing.
4. 50 μL aliquots of the standards, quality control and unknown were pipetted in duplicate in the corresponding tubes. Zero standard was used for NSB tubes.
5. Using Eppendorf repeating dispenser, 500 μL ^{125}I triiodothyronine solution was put in each tube.
6. 500 μL NSB reagent was dispensed in NSB tubes.
7. 500 μL Amerlex - M T_3 reagent was dispensed in all other tubes except TC.
8. All tubes were vortexed and incubated 1 hour at 37°C.
9. Rack was placed on Amerlex - M separator base. It was ensured that all tubes were touching the separator and then left for 15 minutes.
10. Tubes were decanted, and the separator was kept inverted and place on absorbent pad. Drained for 5 minutes. Excess liquid was removed from rim of tubes with Q-tip.
11. Counted on γ counter for 1 minute using Program 9.

AMERLEX - M T$_3$

Pipet as follows:

Tube No.	1 2	3 4	5 6	7 8	9 10	11 12	13 14	15 16	17 18	19 20	21 22	23 24
0.05 mL	TC None	Blank Std A 0	Refer Std A 0	Std B 0.5	Std C 1.5	Std D 3	Std E 6.0	Std F 12.0	QC	QC	QC	Unknown
0.5 mL	^{125}I T$_3$ reagent											
0.5 mL	None	NSB Reagent	None									
0.5 mL	None		Amerlex - M T$_3$ antibody suspension									

Vortexed, incubated at 37°C for 60 minutes. Separated for 15 minutes using Amerlex - M separator. Decanted, blotted. Drained 5 minutes. Counted 1 minute on γ counter prog. 9.

Reference Range

Normal Range 0.80 - 2.71 nmol/L.

References

1. Kodak Clinical Diagnostics. Amerlax-MT3 RIA Kit package insert, 1992. (19 references included)

II.D. Subclinical Effects Markers

II.D.i. Chromosomal Aberrations

From the blood sample collected from each participant in the field, one millilitre of whole blood was used for the analysis of chromosomal aberrations. Chromosomal aberration analysis was performed using methods that adhere to the Health Protection Branch Genotoxicity Guidelines. All procedures prior to cell fixation were performed using biological containment facilities. All unfixed materials were autoclaved prior to disposal.

Cell Culture

Whole blood (0.3 mL), from each participant, was added to T25 tissue culture flasks containing 5 mL McCoy's 5A medium supplemented with antibiotics, fetal calf serum, and phytohemagglutinen (all GIBCO). The cultures were then incubated at 37°C in an atmosphere containing 5% CO_2 and high humidity for 48 - 52 hours. Two hours before harvesting the cells, 0.1 μg/ml colcimid (GIBCO) was added to the cultures.

Cell Harvest

The cells were harvested in the following manner:

1. The cell cultures were transferred to 15 mL capped centrifuge tubes and centrifuged at 1000 g for 6 minutes. The supernatant was decanted and the cells were resuspended.

2. Eight (8) mL of warm (37°C) 0.75 M KCl was then added to the resuspended cells and mixed vigorously by drawing the cell suspension in and out of a pipet several times.

3. The suspended cells were incubated at 37°C for 12 minutes then centrifuged at 1000 g for 6 minutes. The supernatant was decanted and the cells resuspended.

4. Five (5) mL of freshly-prepared 3:1 ethanol-acetic acid fixative were added, a few drops at a time at first, with mixing between additions. This was then let to stand at room temperature for at least 15 minutes.

5. After standing for at least 15 minutes, the cells were centrifuged at 1000 g for 6 minutes. The supernatant was decanted and the cells resuspended.

6. Five (5) mL of fixative was added, and the resuspended cells were centrifuged at 1000 g for 6 minutes. Again the supernatant was decanted and the cells resuspended.

7. The previous step was repeated at least once more.

Slide Preparation

The final cell suspension was diluted to the appropriate cell density with fixative or acetic acid. The diluted cell suspension was then dropped onto glass slides and air dried. The cells on the glass slide were stained with 1% Giemsa in pH 6.8 buffer and then rinsed with distilled water.

Chromosomal Aberration Analysis

All slides were blind coded. For each culture, 100 metaphase cells were analysed for structural and numerical aberrations. The location of each cell and the number and type of aberrations were recorded. All cell locations and analyses were recorded electronically on the Cytoscan automated metaphase finder. This allowed the analyses to be reviewed if necessary.

Reference

1. International Programme on Chemical Safety. Environmental Health Criteria 46: Guidelines for the Study of Genetic Effects in Human Populations. World Health Organization, Geneva, 1985.

II.D.ii. D-glucaric Acid in Urine

An isocratic HPLC procedure was used for determining urinary D-glucaric acid in human urine.

Equipment

Varian 9095 autosampler (Varian Canada, Inc. Georgetown, Ontario, Canada) equipped with a Valco V1C1 valve;

75 x 300 mm Aminex HPX-87H column (Bio-Rad Laboratories, Ontario, Canada) with a Micro-Guard Cation-H guard column;

Waters M-45 pump (Millipore Corporation, Milford, USA) set at 0.5 mL/min;

Shimadzu SPD-6AV UV detector set at 210 nm, and full deflection at 0.01 absorbance;

Goerz Metrawatt SE120 chart recorder (Fisher Scientific Co.) set at 30 cm/h.

Reagents

Mobile phase: 0.004 N H_2SO_4, at room temperature;

Glucaric acid, D-glucuronic acid and Glucuronolactone were obtained from Sigma Chemical Co. and L-ascorbic acid was obtained from BDH Chemicals. Affi-Gel 601 was obtained from Bio-Rad Laboratories;

All other chemicals were reagent grade obtained from Fisher Scientific Co., or Sigma Chemical Co.

Specimen Preparation

1. In a 12 x 75 mm polystyrene tube, 0.2 mL of 1 M potassium phosphate buffer and 100 mg of Affi-Gel 601 was added to 1.8 mL of urine or standards (D-glucaric acid in 0.1 M potassium phosphate, pH 7.0).
2. The mixture was vortexed five times over a 20 minute interval at room temperature.
3. The tube was then centrifuged (5 minutes at 3000 g) and the supernatant was discarded.

4. Two mL of 80 mM potassium phosphate-20 mM boric acid buffer, pH 7.0 was added to the gel sediment and vortexed thoroughly, followed by centrifugation. The supernatant was discarded.

5. The washing and centrifugation step was repeated one more time.

6. The washed gel was then extracted twice with 0.75 mL of 0.1 M HCl by mixing and centrifugation as described above.

7. The first and second urine extracts were pooled and neutralized by adding 30 μl of 5 N NaOH.

HPLC Procedure

Ten (10) μl of urine was injected into the HPX-87H column and eluted with 0.004 N H_2SO_4 at 0.5 mL/minute and RT. The elution peaks were monitored at 210 nm and recorded with a Goerz Metrawatt SE120 chart recorder (Fisher Scientific Co.) set at 30 cm/hour.

Calculation of Results

A 100 μM standard and a normal control accompanied each batch of specimen runs. The urine concentration of D-glucaric acid (μM) for each specimen was calculated from the D-glucaric acid peak height (absorbance) and the peak height of the 100 μM standard.

Quality Assurance and Quality Control

D-glucaric acid standards (25, 50, 100, 200 and 800 μM) were prepared fresh and treated with Affi-Gel 601 and then analysed by HPLC as for the urine extracts. The standard curve should have been linear to 800 μM. A complete standard curve was run *a)* at the beginning of a new series of measurements, *b)* when a new HPLC column was used or after an old column had been rejuvenated and *c)* when a new pump, UV detector, or recorder was used. A supply of normal (about 70 μM) urine extracts was kept at -70°C and each run was accompanied by this control. The absorbance (peak height) of each control should not have varied by more than 10 % from the anticipated absorbance.

Limit of Detection

The method's limit of detection (L.O.D.) was 10 μmoles D-glucaric acid (approximately 7 μmoles/g creatinine).

Reference Range

Urinary D-glucaric acid concentrations in normal adults range from 15 to 89 μmoles/g creatinine (see reference below).

References

1. Poon R., Villeneuve D.C., Chu I., Kinach R. HPLC determination of D-glucaric acid in human urine. Journal of Analytical Toxicology, 17:146-150, 1993.

II.D.iii. Porphyrins in Urine

Porphyrins in urine were determined by high performance liquid chromatography (HPLC).

Materials and Equipment

Column:	Perkin-Elmer HS-3 C-18 3x3 (cat # 0258-0164)
Pump:	Perkin-Elmer Series 4 Liquid Chromatograph
Autoinjector:	Bio-Rad AS100
Detector:	Perkin-Elmer LS-4 Fluorescence Spectrometer, Excitation = 398 nm, Emission = 624 nm

Chromatography Conditions

Mobile phase = 13.8 gm/L $NaH_2PO_4 \cdot H_2O$, pH 3.0 with H_3PO_4. Linear gradient in 2.0 minutes from 30% MeOH to 100% MeOH, hold 3 minutes, linear return in 0.5 minutes to 30% MeOH. Total run time = 9 minutes.

Preparation of Standards

Aliquots were stored in a freezer at -70°C. These were thawed slowly in the dark. 1.0 mL aliquots of standards were acidified to pH ≤ 1 with 25 µL 6M HCl (pH was checked with pH paper). An aliquot of the "porph-free" urine which was used to make up the standards was included. The aliquot was vortexed and a 1 cc tuberculin syringe was used to pass the acidified standards through a Lida 0.45 µm PVDF filter (CSC Inc., Montreal, # 9509-06) into the autoinjector vial (Bio-Rad # 223-9500). The vial was capped (Bio-Rad # 167-0645) and placed in queue for injection of 10 µL.

a. Porphyrin-free urine

A random urine sample (100 mL) was brought to pH 4.5 with glacial acetic acid. Filtered through Whatman paper, and filtered through a 0.45 mm nylon membrane (Fisher # N04SP04700 or equivalent). The porphyrin-free status was confirmed by acidification to pH ≤ 1, filtration, and injection into HPLC.

b. Stock Standard

"Porphyrin Acid Chromatographic Kit" (product # CMK-1A) was obtained from Porphyrin Products, Logan, Utah.

Each vial contained 10 ± 1 nanomoles of URO-, HEPTA-, HEXA-, PENTA-, COPRO-, and MESO- porphyrins.

To one vial was added 1.0 mL 6.0 M HCl and vortexed vigorously. An additional 1.5 mL of HPLC-grade water was added to obtain:

Stock concentration = 20 nmol/5mL = 4.0 nmol/mL = 4.0 pmol/µL = 4000 pmol/mL.

The "porphyrin-free" urine was added with the stock standard to obtain six levels of working standards:

Desired [] working STD	req'd dilution	total volume	req'd vol stock
300 pmol/mL	13.3333	10 mL	750 µL
200	20	10	500
100	40	10	250
50	80	10	125
25	160	10	62.5
10	400	10	25

1.0 mL were aliquoted into 1.5 mL microfuge tubes and stored in freezer. One set was thawed per analysis day and treated as for any sample.

Quality Controls

Bio-Rad Lypochek Quantitative Urine Controls Levels 1 and 2, reconstituted in HPLC-grade H_2O, was used as Quality Controls. The solution was stable up to five days when stored at 4°C. Subsequent preparation for analysis was the same as for samples. The Quality Controls were run immediately after the standards, after every eight samples, and at the end of the run.

Sample Preparation

A 1.0 mL aliquot of urine was placed in a 4.5 mL polypropylene tube (Sarstedt # 57.512). 25 µL of 6M HCl was added and then vortexed after the foaming had subsided. The pH was checked to make sure that it was below 1. The sample was then filtered in the same manner as described for the standards (above) and placed in queue for injection of 10 µL.

Data Analysis

Using a Dionex AI-450 Advanced Computer Interface (software version 3.31), sample porphyrin concentrations were determined from linear standard curves with forced zero intercept.

Reference Range

Uroporphyrin:	0 - 42 nmol/day
Hepta, hexa, pentaporphyrin:	<25 nmol/day
Coproporphyrin:	0 - 245 nmol/day

II.E. Confounding Exposure Markers

III.E.i. Cotinine in Urine

The Serex Coti-Traq assay was used to determine cotinine in the urine samples provided by the participants. It is a competitive immunoassay utilizing ELISA technology to provide a spectrophotometric indicator of the amount of cotinine in samples of body fluids. Since this is a competitive assay, the lower the concentration of cotinine in the sample or standard, the higher the absorbance found. The range of the assay has been set to 0.1 - 1 µg/mL. Samples with higher concentrations were diluted and reassayed.

Materials and Equipment

Pipets or pipettors: 10, 25, 50, 100, 125, and 200 µL;

Volumetric pipets: 1 and 6 mL;

Molecular Devices Thermomax Microplate Reader.

Reagents

Three Coti-Traq strip well plates;

Three Coti-Traq Antiserum, 6 mL each, lyophilized;

Three Coti-Traq Conjugate, 6 mL each, lyophilized;

Five Cotinine Standards, 0, 0.01, 0.1, 0.5, and 1.0 µg/mL, 1.0 mL each;

Three Phosphate Buffered Saline (PBS) tablets;

One TMB substrate, (20x concentrate), 5 mL;

One stop solution (2N sulphuric acid), 15 mL.

Reagent Preparation

PBS: Individual tablets were dissolved in 200 mL of distilled oe deionized water. Each plate required 110 mL. PBS reagents were stored at 2 - 8 °C after reconstitution, and were not used more than 21 days afterward.

Standards: The seal and stopper was removed from each vial and the standard was reconstituted with exactly 1.0 mL of deionized water. The stopper was replaced and the contents swirled gently to mix. Fifteen minutes for complete reconstitution were allowed before use. The vials were refrigerated after each use.

Antiserum and Conjugate: The seals and stoppers of the Coti-Traq Antiserum and Conjugate bottles were removed, and each were reconstituted with exactly 6.0 mL of deionized water. The stoppers were replaced and the contents swirled gently to mix. Fifteen minutes for complete reconstitution were allowed before use.

TMB Reagent: The seal and stopper of the TMB Reagent bottle was removed and the reagent was diluted 1:20 with deionized. The diluted TMB reagent was stable for five working days if protected from light.

Specimen Preparation

1. The plate and reagents were removed from refrigeration and allowed to come to room temperature.
2. 125 µL of PBS was added to each well using a multichannel pipettor.
3. 10 µL of samples (1:5 dilution) and standards were added to the plate wells. Samples and standards were run in duplicate. Separate pipet tips were used for each sample or standard.
4. 50 µL of reconstituted Coti-Traq Antiserum was added to each well.
5. The plate was incubated for 60 minutes at room temperature (20 - 25 °C).
6. After incubation, the plate was inverted to dump out the well contents. The plate was washed four times by adding 200 µL of PBS per well. The inverted plate was tapped vigorously on a clean towel to ensure complete removal of liquid.
7. 150 µL of PBS and 50 µL of reconstituted Conjugate was added to each well and the plate was then incubated for 30 minutes at room temperature..
8. After incubation, the plate was washed four times as directed in step 6 above.
9. 150 µL of diluted TMB reagent was added to each plate well and the plate was incubated for 15 minutes at room temperature.
10. The reaction was stopped by adding 50 µL of stop solution (2N sulphuric acid) to each well. The plates were tapped to mix the contents thoroughly and then let stand for an additional 15 minutes before reading absorbance.

11. Absorbance was measured at 450 nm. A standard curve was prepared from the readings obtained with the standards and the concentration of the samples were determined from the standard curve.

Quality Assurance and Quality Control

Each sample was run in duplicate. Analysis was repeated if the difference between the duplicates was greater than 10%. Standards were analysed and standard curves were generated for each batch of samples run.

Limit of Detection

To be supplied by R. Poon.

Reference Range

Using gas liquid chromatography analysis for urinary cotinine, Jarvis (1987) used the value of 0.023 µg/ml (23 ng/ml) as the cut-off where higher values were considered to be from active smokers. Values below this cut-off were considered to be from non-smokers or from sidestream exposure.

Using gas liquid chromatography analysis for urinary cotinine, Wall et al. (1988) reported the following results in smokers and nonsmokers:

	Urinary Cotinine (ng/ml)	Urinary Cotinine ÷ Urinary Creatinine
Nonsmokers	6.0	3.6
Passive smokers	9.2	6.3
Active smokers (≤ 10 cgts/day)	646.8	673.4
Active smokers (>10 cgts/day)	1100.7	1325.5

Reference

1. Serex INC. Coti-Traq Test, 1992.

2. Jarvis M.J. American Journal of Public Health, 77:1435, 1987.

3. Wall M.A. et al. American Journal of Public Health, 78(6):699-701, 1988.

III. ANALYTICAL QUALITY CONTROL CHECKS

A. Organochlorine Pesticides and PCB Analysis in Blood

Human plasma, supplied by the Centre de Toxicologie du Québec, was used to prepare samples spiked with reference material for use as analytical quality control (QC) checks during the study.

Reference Materials

Reference materials for spiking plasma samples were supplied by Le Centre de Toxicologie du Québec. These included reference material for the following contaminants: Hexachlorobenzene, p,p' DDE, Dieldrin, Mirex, Oxychlordane, PCB congeners 138, 153, 180, 183, and 187.

Human Plasma

Approximately 200 mL of fresh human plasma was provided by Le Centre de Toxicologie du Québec. The plasma was previously analyzed by the supplier to ensure that its levels of contaminants were low.

Spiking Scheme

The following spiking scheme was used to prepare the analytical quality control check samples:

Compound	L.O.Q. (µg/L)	Spiked Level (µg/L)
Hexachlorobenzene	0.05	0.15
p,p' DDE	0.05	5.0
Dieldrin	0.2	0.1
Mirex	0.05	0.125
Oxychlordane	0.05	0.125
PCB 138	0.05	0.75
PCB 153	0.05	1.0
PCB 180	0.05	0.75
PCB 183	0.05	0.15
PCB 187	0.05	0.5

Not all target compounds were included in the spiking scheme. The contaminants not included in the preparation of the analytical QC check samples were p,p' DDT, alpha-chlordane, gamma-chlordane, transnonachlor, heptachlor epoxide, total PCB as Aroclor 1260, and PCB congeners 28, 52, 99, 101, 105, 118, 128, 156, 170. The analytical laboratory considered the above spiking scheme as representative of the target contaminants expected in human samples and was, therefore, adequate as a check of the laboratory's analytical results during the project.

Preparation of Quality Control Check Samples

A total of 25 plasma samples were prepared as analytical QC check samples. Each sample consisted of a total volume of 5 mL of plasma and the appropriate spiking material. The QC check samples were prepared in 7 mL Supelco vials (no. 2-3248) identical to the samples collected in the field. Of these 25 samples, 5 plasma samples were not spiked and 20 plasma samples were spiked according to scheme in the table above. The spiking of the plasma samples was set at a level approximately midway between the limit of quantification (L.O.Q.), and average levels of contaminants expected in the population. These spiking levels were also chosen in order not to replicate the spiking levels used in a previous analytical audit of the laboratory.

In this laboratory QC check, dieldrin was spiked (0.1 µg/L) at a level below the limit of quantification (0.2 µg/L). In the laboratory audit performed prior to the study, the L.O.Q. for the analytical procedure followed at the time was 0.05 µg/L. In the interim between the laboratory audit and the initiation of this study, the laboratory modified its procedures so that a greater range of contaminants could be analyzed. However, as a result, the L.O.Q. for dieldrin increased from 0.05 µg/L to 0.2 µg/L. The spiking solutions had already been prepared by the time the modified procedure was supplied by the laboratory. Rather than preparing new solutions it was decided that, for dieldrin, plasma could be spiked at 0.1 µg/L which was also at the method's limit of detection and therefore useful in assessing the quality of analysis.

Labeling and Handling of Quality Control Check Samples

Dummy identification numbers and sampling dates were predetermined for all analytical QC check samples. All vials containing these samples were labelled in the same fashion as samples collected from the study participants. Each week, four or five of these samples were included with the regular shipment of samples to the laboratory. A BLOOD SPECIMEN REPORT FORM was filled out for each sample and accompanied it to its destination.

Results

PCBs and Organochlorine Pesticides in Plasma: Quality Control Check Samples

Compound	A	B	C	E
	Spiked Level (µg/L)	Mean of Non-spiked Samples (µg/L) n = 5	Mean of Spiked Samples (µg/L) n = 20	Corrected Ratio** $\frac{C - B}{A}$ n = 20
Hexachlorobenzene	0.15	0.012* (1)	0.154 (20)	0.95
p,p' DDE	5.0	0.106 (5)	5.40 (20)	1.06
Dieldrin	0.1	0.05* (0)	0.067* (5)	0.17
Mirex	0.125	0.01* (0)	0.132 (20)	0.98
Oxychlordane	0.125	0.01* (0)	0.133 (20)	0.98
PCB 138	0.75	0.02 (5)	0.723 (20)	0.94
PCB 153	1.0	0.03 (5)	0.924 (20)	0.89
PCB 180	0.75	0.022 (5)	0.714 (20)	0.92
PCB 183	0.15	0.01* (0)	0.144 (20)	0.89
PCB 187	0.5	0.014* (2)	0.474 (20)	0.92

The values in parentheses indicate the number of samples measured above the detection limit.

* In cases where the measured values were below the detection limit, the means were calculated using values at one-half of the detection limit. For example, for the non-spiked PCB 187 samples, three samples were below the limit of detection of 0.02 µg/L. Therefore, 0.01 µg/L (one-half L.O.D.) was used as the default value for these samples. The other two samples were each measured at 0.02 µg/L. Thus the overall mean was calculated at 0.014 µg/L.

** A Corrected Ratio of 1.0 represents an exact agreement between the amount of contaminant measured and the amount of contaminant added to spike the plasma QC check sample.

The laboratory's own quality control report for analyses performed during the life of the study is included on the following pages.

Centre de Toxicologie du Québec

DÉTERMINATION DES BPC ET DES PESTICIDES ORGANOCHLORÉS DANS LE PLASMA, DANS LE CADRE DU PROJET "THE GREAT LAKES ANGLERS PILOT EXPOSURE ASSESSMENT STUDY"

CONTRÔLE DE QUALITÉ

Liliane Ayotte Ferron, chimiste
Sylvie Gagnon, technologiste
Francine Labrecque, technologiste
Mario Marchand, technologiste
Évelyne Pelletier, technologiste

Février 1993

Le Centre Hospitalier de l'Université Laval – 2705, boul. Laurier, Sainte-Foy, Québec. G1V 4G2
TÉL.: (418) 654-2254 (418) 654-2100 (laboratoire)
TÉLÉCOPIEUR/FAX (418) 654-2754

TABLE DES MATIÈRES

Page

1. Protocole de contrôle de qualité 1

2. Tableaux des "K" pour l'étalon de référence à 0.5 μg/L 3, 4

3. Tableaux des résultats obtenus pour le contrôle "Serum NBS + organochlorés" . . 5, 6

4. Description des Notes 7

5. Description du diagramme C/P 8

6. Diagrammes C/P

BPC . . . . 9 à 19

Pesticides organochlorés . . . . 20 à 29

PROTOCOLE DE CONTRÔLE DE QUALITÉ LORS DES ANALYSES DE BPC ET PESTICIDES ORGANOCHLORÉS

En raison de la sensibilité attendue (limite de quantification = 0.05 μg/L) le dosage des BPC et des pesticides organochlorés exige un contrôle rigoureux de toutes les étapes. Afin d'assurer la fiabilité des résultats, le présent protocole doit être scrupuleusement respecté.

1. Traitement de la verrerie

Toute la verrerie doit être nettoyée selon la méthode E-347-B (no. 10, p. 5).

2. Préparation des réactifs

2.1 Tous les réactifs doivent être préparés et purifiés selon la méthode E-347-B (no. 9, p. 4).

2.2 Tous les réactifs doivent être datés et réservés exclusivement à ces dosages. À chaque fois qu'un réactif change, un blanc est extrait afin de vérifier les contaminations.

2.3 Tous les changements concernant les réactifs doivent être notés.

3. Préparation des étalons

3.1 Toutes les nouvelles solutions étalons doivent être validées (p/r à l'ancienne).

3.2 Tous les changements concernant les solutions étalons doivent être notés.

4. Étalonnage

4.1 Pour chaque série de dosages préparer:

- Un étalon à 0.5 μg/L (congénères BPC + organochlorés) non extrait dans l'hexane,
- Un étalon à 0.5 μg/L extrait dans l'eau,
- Un étalon à 0.5 μg/L extrait dans le plasma.

4.2 L'étalon non extrait est injecté au début de la séquence et est utilisé pour vérifier la sensibilité des détecteurs.

4.3 L'étalon extrait dans l'eau est utilisé comme calibrateur pour le dosage de tous les produits sauf: hexachlorobenzène, aldrin et pp'-DDT.

4.4 L'étalon extrait dans le plasma est utilisé comme calibrateur pour le dosage de l'hexachlorobenzène, de l'aldrin et du pp'-DDT et pour vérifier les pourcentages de recouvrement.

4.5 Les données (rapports des aires) provenant des étalons extraits sont compilées dans des tableaux.

5. Contrôles

5.1 À chaque série de dosages, analyser le contrôle de concentration connue, Serum NBS # 1589 (dilué 1/10) + organochlorés à 0.4 μg/L.

5.2 À chaque série de dosages, analyser le plasma normal Otw # 2.

5.3 Les résultats des contrôles sont compilés dans un tableau et entrés sur les diagrammes de contrôle C/P à l'aide du programme informatisé "Harvard Graphic".

5.4 La tolérance maximale acceptée est ± 2σ.

Centre de Toxicologie du Québec

CONTRÔLE DE QUALITÉ

Étalon de référence à 0.5 μg/L
Tableau des K*

Date	No de série	Congénères													
		28	52	99	101	105	118	128	138	153	156	170	180	183	187	Notes
92-11-23	OC-001	0.41	0.44	0.73	0.66	0.68	0.65	1.01	0.81	0.84	1.01	1.09	0.99	0.99	0.87	1
92-11-25	OC-002	0.32	0.31	0.51	0.53	0.69	0.56	0.94	0.75	0.68	1.08	1.15	0.87	0.85	0.79	
92-11-26	OC-003	0.42	0.51	0.59	0.63	0.72	0.68	1.01	0.89	0.82	1.08	0.96	1.06	1.08	0.83	
92-12-01	OC-004	0.32	0.31	0.52	0.60	0.71	0.64	1.02	0.87	0.80	1.08	1.11	1.00	0.99	0.85	2
92-12-07	OC-005	0.38	0.34	0.65	0.64	0.67	0.62	0.99	0.81	0.77	0.96	1.03	0.91	0.90	0.94	3, 4
92-12-08	OC-006	0.43	0.41	0.68	0.68	0.62	0.61	0.92	0.82	0.80	0.79	0.91	0.90	0.96	0.85	
92-12-14	OC-007	0.41	0.39	0.68	0.66	0.70	0.64	1.04	0.78	0.78	1.04	1.06	0.92	0.91	0.85	
92-12-16	OC-008	0.41	0.39	0.68	0.66	0.70	0.64	1.04	0.78	0.78	1.04	1.06	0.92	0.91	0.85	5
92-12-21	OC-009	0.37	0.35	0.69	0.62	0.61	0.56	1.07	0.84	0.79	1.11	1.21	1.01	0.93	0.86	
92-12-21	OC-010	0.44	0.41	0.73	0.68	0.60	0.57	0.97	0.85	0.83	0.81	1.06	1.11	1.00	0.93	
93-01-04	OC-013	0.35	0.40	0.85	0.66	0.75	0.73	1.21	0.85	0.83	1.26	1.29	0.98	0.96	0.89	6,7,8,9
93-01-14	OC-014	0.28	0.21	0.53	0.43	0.62	0.53	1.12	0.72	0.64	1.16	1.21	0.88	0.80	0.71	
93-01-06	OC-015	0.51	0.36	0.79	0.61	0.75	0.66	1.21	0.82	0.77	1.24	1.30	0.95	0.91	0.77	
93-01-07	OC-016	0.39	0.30	0.71	0.57	0.67	0.60	1.15	0.78	0.73	1.20	1.26	0.91	0.87	0.79	
93-01-11	OC-017	0.42	0.26	0.66	0.55	0.68	0.60	1.14	0.77	0.70	1.21	1.27	0.90	0.85	0.76	4
93-01-13	OC-018	0.58	0.44	0.85	0.69	0.79	0.73	1.25	0.86	0.85	1.30	1.31	0.97	0.95	0.88	
93-01-21	OC-019	0.50	0.39	0.73	0.67	0.74	0.67	1.06	0.89	0.84	1.19	1.20	0.95	0.95	0.86	4, 10
93-01-21	OC-020	0.37	0.30	0.67	0.58	0.71	0.64	1.10	0.80	0.75	1.22	1.23	0.97	0.88	0.77	11
93-01-25	OC-021	0.47	0.36	0.66	0.62	0.70	0.62	0.94	0.77	0.75	1.06	1.08	0.88	0.86	0.75	12
93-01-26	OC-022	0.50	0.36	0.65	0.61	0.68	0.60	0.98	0.77	0.73	1.03	1.08	0.89	0.86	0.79	
93-01-27	OC-023	0.52	0.38	0.74	0.69	0.71	0.68	1.01	0.82	0.79	1.09	1.13	0.93	0.93	0.82	
93-01-28	OC-024	0.48	0.33	0.64	0.58	0.64	0.67	1.01	0.78	0.72	1.09	1.10	0.88	0.84	0.78	
93-02-01	OC-025	0.59	0.40	0.73	0.67	0.76	0.70	1.03	0.81	0.80	1.14	1.05	0.94	0.90	0.80	4

$$* \; K = \frac{\text{aire du produit à doser}}{\text{aire de l'étalon interne}}$$

Centre de Toxicologie du Québec

CONTRÔLE DE QUALITÉ

Étalon de référence à 0.5 μg/L
Tableau des K*

Date	No de série	β-BHC	α-chlor dane	γ-chlor dane	pp'-DDE	pp'-DDT	Dieldrin	Hepta chlor époxide	Hexa chloro benzène	Mirex	Oxy chlor dane	Trans nona chlor
92-11-23	OC-001	0.28	0.75	0.54	0.49	0.14	0.45	0.51	0.75	0.68	0.72	0.65
92-11-25	OC-002	0.23	0.59	0.54	0.40	0.21	0.31	0.44	0.46	0.61	0.59	0.64
92-11-26	OC-003	0.28	0.61	0.59	0.61	0.16	0.36	0.52	0.54	0.81	0.59	0.74
92-12-01	OC-004	0.27	0.58	0.58	0.46	0.13	0.42	0.46	0.45	0.67	0.57	0.68
92-12-07	OC-005	0.25	0.53	0.56	0.48	0.14	0.32	0.46	0.66	0.67	0.52	0.66
92-12-08	OC-006	0.26	0.60	0.60	0.51	0.12	0.28	0.41	0.70	0.65	0.55	0.70
92-12-14	OC-007	0.26	0.64	0.59	0.56	0.11	0.40	0.51	0.77	0.65	0.62	0.70
92-12-16	OC-008	0.26	0.64	0.59	0.56	0.11	0.34	0.53	0.77	0.65	0.62	0.70
92-12-21	OC-009	0.28	0.72	0.64	0.50	0.11	0.55	0.77	0.65	0.58	0.72	0.97
92-12-21	OC-010	0.29	0.68	0.67	0.52	0.13	0.36	0.48	0.64	0.62	0.70	0.72
93-01-04	OC-013	0.30	0.78	0.59	0.51	0.34	0.49	0.36	0.91	0.71	0.55	0.67
93-01-14	OC-014	0.22	0.59	0.45	0.37	0.28	0.35	0.30	0.51	0.53	0.46	0.49
93-01-06	OC-015	0.29	0.78	0.57	0.44	0.31	0.40	0.35	0.66	0.66	0.60	0.68
93-01-07	OC-016	0.31	0.71	0.54	0.44	0.29	0.37	0.34	0.72	0.62	0.58	0.60
93-01-11	OC-017	0.23	0.66	0.50	0.42	0.23	0.40	0.35	0.70	0.58	0.46	0.53
93-01-13	OC-018	0.39	0.93	0.66	0.56	0.25	0.41	0.38	0.65	0.76	0.50	0.73
93-01-21	OC-019	0.33	0.81	0.62	0.48	0.27	0.37	0.36	0.68	0.80	0.66	0.74
93-01-21	OC-020	0.31	0.76	0.58	0.45	0.26	0.40	0.35	0.54	0.73	0.60	0.66
93-01-25	OC-021	0.33	0.73	0.57	0.47	0.23	0.26	0.32	0.70	0.80	0.43	0.70
93-01-26	OC-022	0.25	0.67	0.56	0.43	0.19	0.25	0.30	0.66	0.71	0.38	0.64
93-01-27	OC-023	0.31	0.73	0.54	0.49	0.20	0.30	0.34	0.77	0.79	0.59	0.70
93-01-28	OC-024	0.25	0.69	0.53	0.42	0.22	0.28	0.35	0.59	0.72	0.45	0.65
93-02-01	OC-025	0.35	0.71	0.59	0.44	0.13	0.30	0.35	0.93	0.69	0.68	0.73

$$* \; K = \frac{\text{aire du produit à doser}}{\text{aire de l'étalon interne}}$$

Notes: Les notes mentionnées sur la page 3 s'appliquent aussi pour ce tableau.

Centre de Toxicologie du Québec

CONTRÔLE DE QUALITÉ

Sérum NBS # 1589 (dilué 1/10) + organochlorés

Biphényles Polychlorés (µg/L)

Date	No de série	Congénères												Notes
		Aroclor 1260	101	105	118	128	138	153	156	170	180	183	187	
92-11-23	OC-001	8.2	0.28	0.29	0.90	0.04	0.75	0.82	0.15	0.38	0.91	0.25	0.57	1
92-11-25	OC-002	8.7	0.35	0.21	1.01	0.05	0.78	0.90	0.14	0.38	1.0	0.27	0.61	
92-11-26	OC-003	7.7	0.26	0.25	0.83	0.03	0.67	0.80	0.16	0.45	0.96	0.23	0.54	
92-12-01	OC-004	8.4	0.36	0.22	0.94	0.04	0.77	0.85	0.17	0.39	0.96	0.27	0.63	
92-12-07	OC-005	7.6	0.26	0.23	0.89	0.04	0.70	0.76	0.17	0.37	0.89	0.22	0.54	3,4
92-12-08	OC-006	8.0	0.36	0.26	0.99	0.04	0.72	0.81	0.19	0.42	0.97	0.24	0.56	
92-12-14	OC-007	8.4	0.31	0.22	0.93	0.04	0.77	0.84	0.15	0.39	0.97	0.26	0.57	
92-12-16	OC-008	8.9	0.33	0.25	1.04	0.04	0.81	0.91	0.16	0.40	1.01	0.27	0.62	5
92-12-21	OC-009	8.0	0.30	0.28	1.12	0.04	0.71	0.82	0.15	0.36	0.84	0.24	0.57	
92-12-21	OC-010	7.8	0.27	0.27	0.95	0.04	0.67	0.83	0.17	0.37	0.81	0.22	0.51	
93-01-04	OC-013	7.9	0.30	0.23	0.87	0.04	0.72	0.79	0.16	0.37	0.92	0.24	0.52	6, 7, 9
93-01-14	OC-014	7.5	0.29	0.21	0.82	0.04	0.68	0.77	0.15	0.38	0.92	0.24	0.53	
93-01-06	OC-015	8.4	0.31	0.23	0.91	0.04	0.75	0.86	0.17	0.37	0.95	0.25	0.61	
93-01-07	OC-016	8.2	0.31	0.24	0.88	0.04	0.74	0.83	0.16	0.38	0.95	0.25	0.58	
93-01-11	OC-017	7.2	0.32	0.22	0.95	0.04	0.72	0.86	0.15	0.35	0.92	0.25	0.57	4
93-01-13	OC-018	7.9	0.33	0.22	0.81	0.04	0.71	0.80	0.15	0.35	0.92	0.22	0.55	
93-01-21	OC-019	7.2	0.28	0.22	0.85	0.04	0.65	0.74	0.15	0.37	0.89	0.21	0.55	4, 10
93-01-21	OC-020	8.0	0.30	0.22	0.86	0.04	0.73	0.81	0.14	0.34	0.87	0.23	0.59	11
93-01-25	OC-021	8.4	0.32	0.24	0.92	0.04	0.76	0.86	0.16	0.39	0.99	0.25	0.63	
93-01-26	OC-022	8.3	0.31	0.24	0.90	0.06	0.75	0.84	0.16	0.41	0.97	0.26	0.58	
93-01-27	OC-023	7.6	0.26	0.22	0.82	0.04	0.69	0.77	0.16	0.39	0.93	0.24	0.56	
93-01-28	OC-024	8.2	0.32	0.24	0.89	0.04	0.72	0.86	0.15	0.38	0.95	0.24	0.60	
93-02-01	OC-025	8.2	0.32	0.21	0.88	0.04	0.74	0.82	0.14	0.39	0.94	0.25	0.60	4

Centre de Toxicologie du Québec

CONTRÔLE DE QUALITÉ

Sérum NBS # 1589 (dilué 1/10) + organochlorés

Pesticides Organochlorés (µg/L)

Date	No de série	β-BHC	α-chlor dane	γ-chlor dane	pp'-DDE	Dieldrin	Hepta chlor époxide	Hexa chloro benzène	Mirex	Oxy chlor dane	Trans nona chlor	Notes
92-11-23	OC-001	0.36	0.40	0.39	0.41	—	0.37	0.38	0.43	0.33	0.37	1
92-11-25	OC-002	0.46	0.47	0.39	0.45	0.37	0.30	0.41	0.43	0.43	0.40	
92-11-26	OC-003	0.38	0.44	0.34	0.36	0.38	0.39	0.40	0.38	0.38	0.36	
92-12-01	OC-004	0.44	0.47	0.38	0.47	0.41	0.30	0.47	0.43	0.43	0.41	2
92-12-07	OC-005	0.36	0.36	0.35	0.38	0.59	0.51	0.41	0.44	0.35	0.34	3, 4
92-12-08	OC-006	0.37	0.47	0.40	0.39	0.44	0.30	0.36	0.44	0.43	0.37	
92-12-14	OC-007	0.42	0.43	0.36	0.36	0.44	0.35	0.42	0.41	0.40	0.34	
92-12-16	OC-008	0.47	0.43	0.37	0.38	0.42	0.37	0.20	0.41	0.39	0.38	5
92-12-21	OC-009	0.42	0.43	0.34	0.43	0.33	0.32	0.39	0.41	0.37	0.35	
92-12-21	OC-010	0.37	0.41	0.31	0.35	0.40	0.33	0.34	0.39	0.34	0.32	
93-01-04	OC-013	0.44	0.47	0.36	0.38	0.32	0.36	0.47	0.40	0.38	0.36	6,7,8,9
93-01-14	OC-014	0.39	0.36	0.32	0.36	0.37	0.42	0.40	0.37	0.39	0.32	
93-01-06	OC-015	0.39	0.38	0.34	0.41	0.40	0.39	0.35	0.43	0.33	0.36	
93-01-07	OC-016	0.39	0.41	0.35	0.40	0.40	0.38	0.48	0.43	0.36	0.36	
93-01-11	OC-017	0.44	0.43	0.36	0.41	0.46	0.49	0.53	0.45	0.32	0.38	4
93-01-13	OC-018	0.32	0.35	0.32	0.34	0.45	0.39	0.44	0.38	0.50	0.33	
93-01-21	OC-019	0.38	0.41	0.33	0.37	0.42	0.40	0.37	0.37	0.37	0.33	4,10
93-01-21	OC-020	0.37	0.39	0.33	0.38	0.47	0.39	0.59	0.39	0.39	0.34	11
93-01-25	OC-021	0.39	0.41	0.38	0.40	0.39	0.40	0.42	0.42	0.46	0.37	12
93-01-26	OC-022	0.43	0.44	0.36	0.42	0.40	0.41	0.30	0.42	0.41	0.37	
93-01-27	OC-023	0.34	0.40	0.37	0.37	0.34	0.35	0.36	0.37	0.32	0.34	
93-01-28	OC-024	0.42	0.43	0.37	0.42	0.38	0.37	0.37	0.43	0.33	0.37	
93-02-01	OC-025	0.37	0.42	0.38	0.41	0.37	0.39	0.41	0.42	0.32	0.36	4

NOTES

1. Évaporation à l'aide de l'évaporateur "Speed-Vac Concentrator" de Savant.

2. Modification de la programmation de température pour doser le dieldrin et l'heptachlorépoxide dans la deuxième élution.

3. Évaporation de la première élution à l'aide de l'évaporateur rotatif "Rotavapor-R".

4. Nouveau Florisil à 0.5%.

5. Nouvelle solution de sulfate d'ammonium.

6. Évaporation à l'aide de l'évaporateur "Speed Vac Plus", modèle SC210A de Savant.

7. Changement de lot pour le Florisil.

8. Changement de système chromatographique (HP-5890-II) pour doser le dieldrin et l'heptachlorépoxide dans la deuxième élution.

9. * Changement de "insert liner" dans l'injecteur du système chromatographique HP-5890-I.

10. Nouvelle solution de sulfate d'ammonium.

11. Changement de lot pour l'hexane, lot # 925448.

12. Modification de la programmation de température sur le système HP-5890-II pour doser le dieldrin et l'heptachlorépoxide dans la deuxième fraction.

* "L'insert liner" influence le comportement du pp'-DDT: la sensibilité du pp'-DDT diminue avec le vieillissement de "l'insert liner". Les limites de détection et de quantification ont été établies en tenant compte de ce facteur: K pour pp'-DDT doit être ≥ 0.1.

DIAGRAMME DE CONTRÔLE (C/P)

Le diagramme de contrôle de qualité que nous utilisons au laboratoire de toxicologie est le diagramme "C/P" décrit par R.A. Nadkarni.

Description du diagramme C/P

Les lectures individuelles sont représentées par des astérisques.

Les lignes de biais (b), d'avertissement (a) et de mesure à prendre (m) sont les lignes 1σ, 2σ et 3σ respectivement.

La ligne des tendances 20% (en pointillés) réduit les données pour démontrer une constance ou une tendance dans la moyenne.

Interprétation du diagramme C/P

La lecture est-elle au-delà de la ligne d'avertissement ou de mesure à prendre?

> Si oui, soyez vigilant.
>
> Si la lecture coupe la ligne d'avertissement, vérifier si toutes les étapes sont sous contrôle.
>
> Si la lecture coupe la ligne de mesure à prendre, une action immédiate doit être prise.

Est-ce que 2 des 3 dernières lectures sont au-delà de la ligne d'avertissement?

> Les deux lectures sont du même côté: les résultats s'éloignent de la valeur cible ou moyenne.
>
> Les deux lectures sont de part de d'autre: la variabilité de la méthode augmente.

La ligne des tendances s'approche ou coupe la ligne de biais?

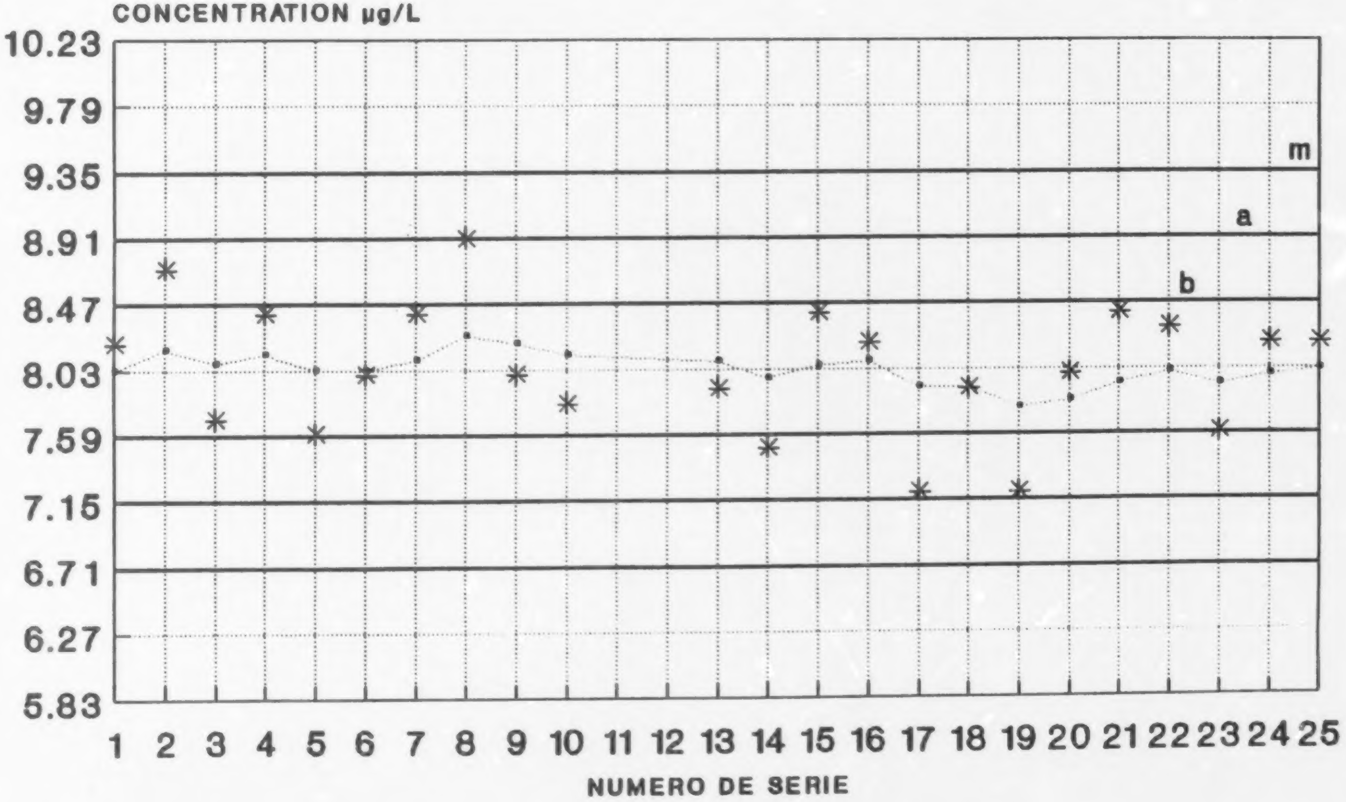
> Si elle traverse la ligne de biais, une action immédiate doit être prise.
>
> Si elle approche la ligne de biais et se situe au ⅔ de la distance, soyez vigilant, vérifiez si la moyenne a changé.

Référence: Nadkarni R.A. **The quest for quality in laboratory.** Anal. Chem. 63(13):675A-682A, 1991.

DIAGRAMME DE CONTROLE C/P

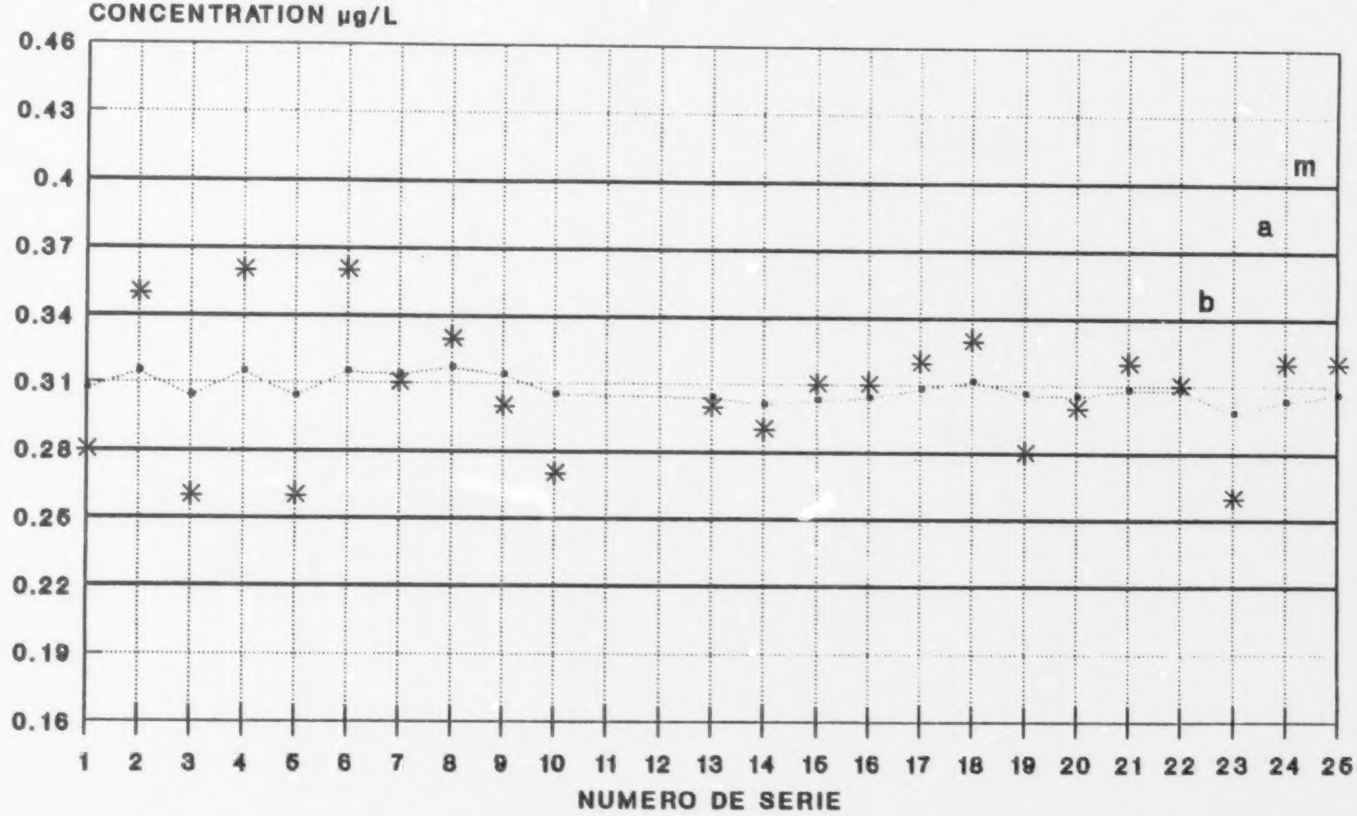
AROCLOR 1260 DANS LE PLASMA

Contrôle NBS 1/10, 921119



PERIODE(23/11/92 - 01/02/93)
MOYENNE = 8.03
SIGMA = .44 C.V.=5.42%

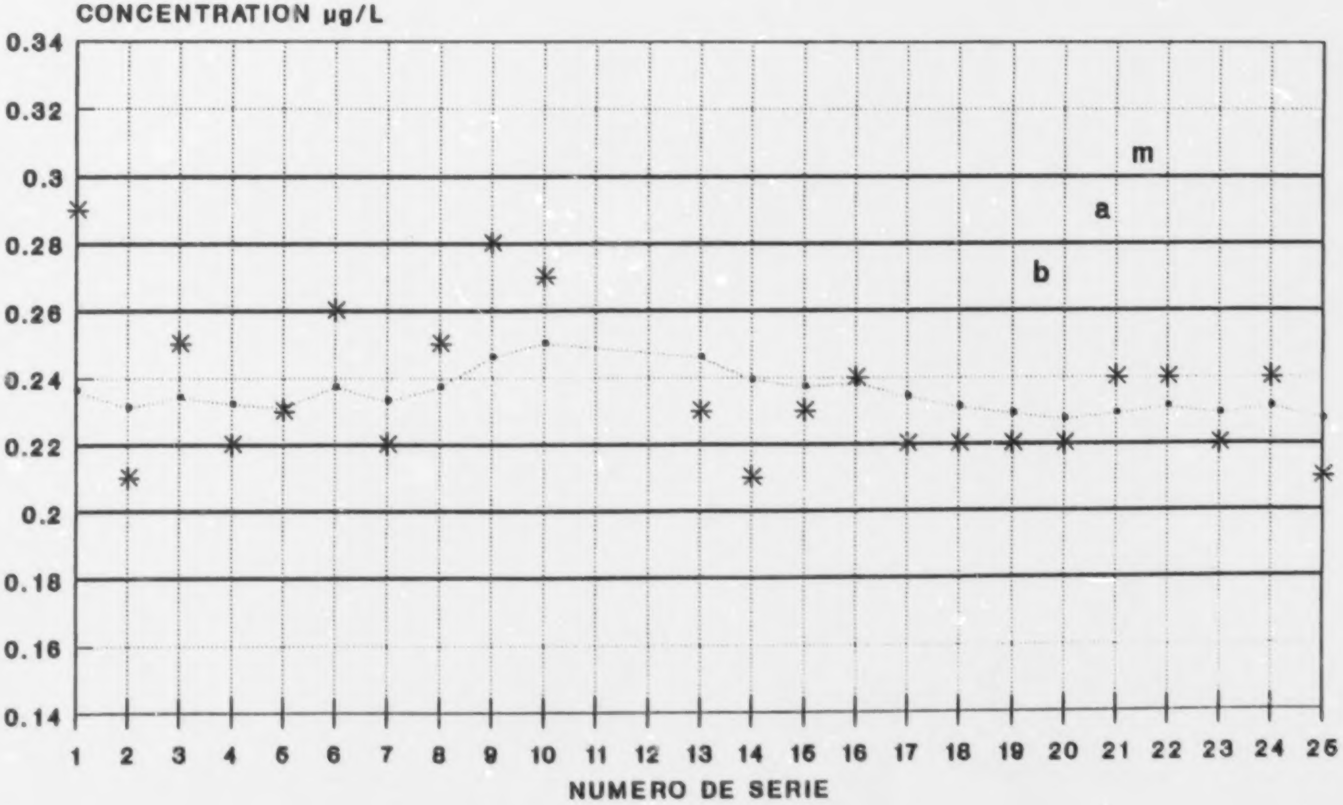
DIAGRAMME DE CONTROLE C/P
BPC #101 DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.31
SIGMA=.03 C.V.=9.62%

DIAGRAMME DE CONTROLE C/P

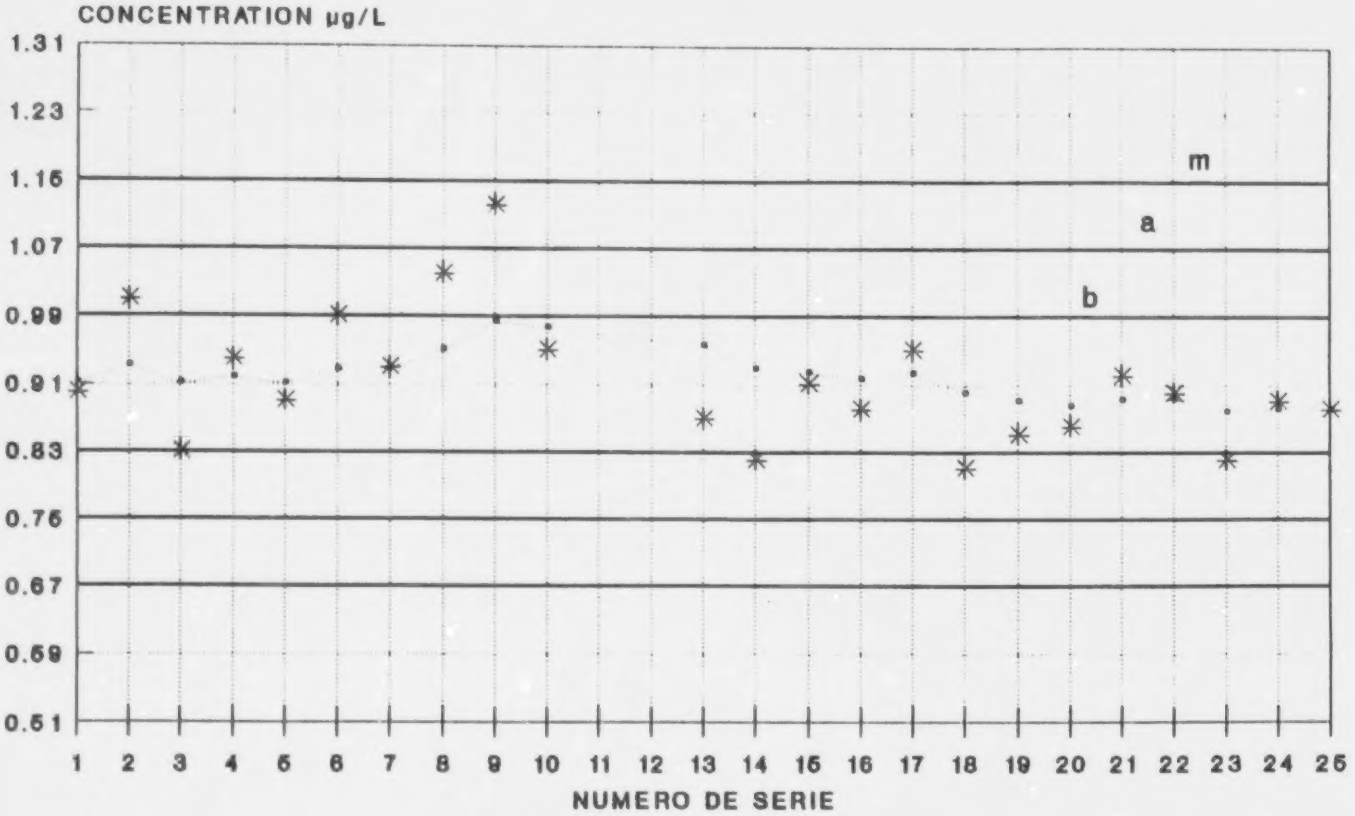
BPC #105 DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.24
SIGMA=.02 C.V.=9.47%

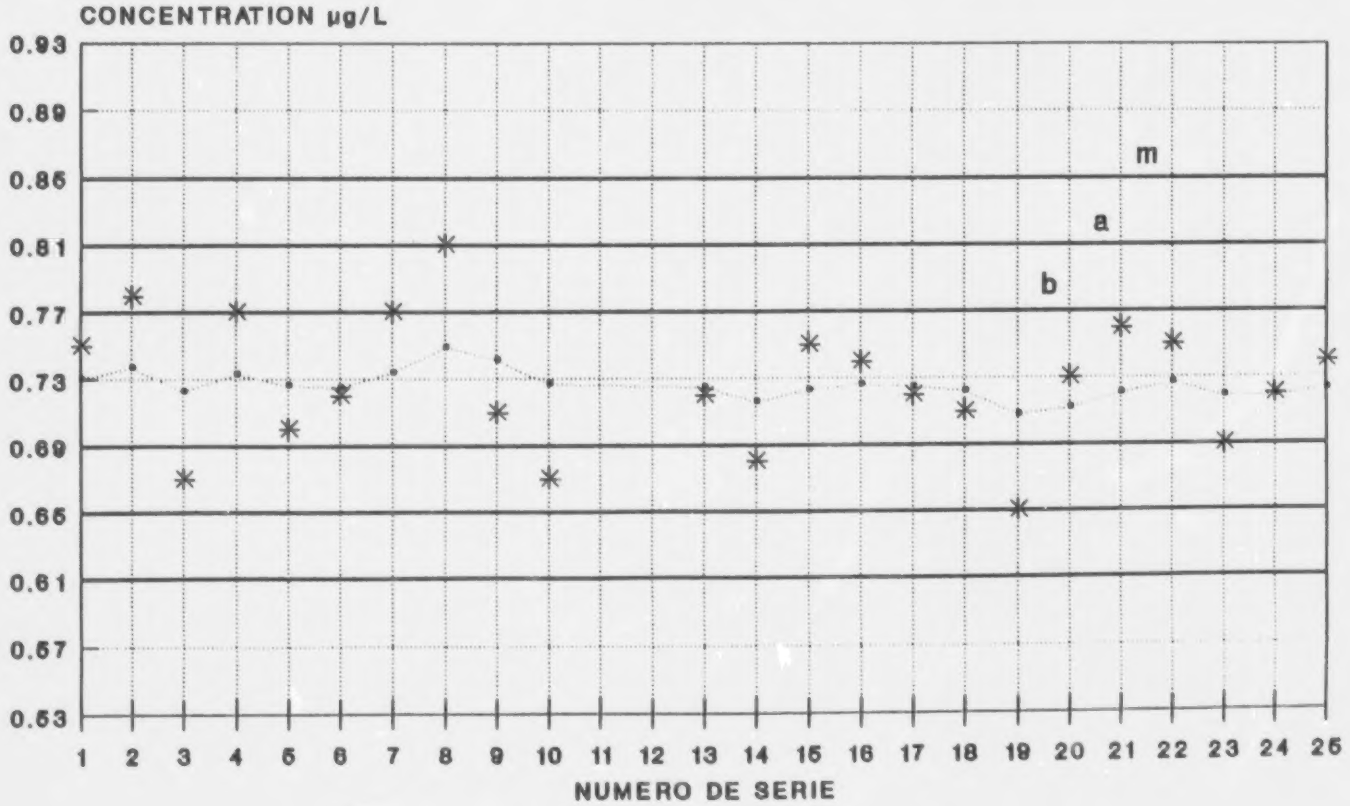
DIAGRAMME DE CONTROLE C/P

BPC #118 DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.91
SIGMA=.08 C.V.=8.27%

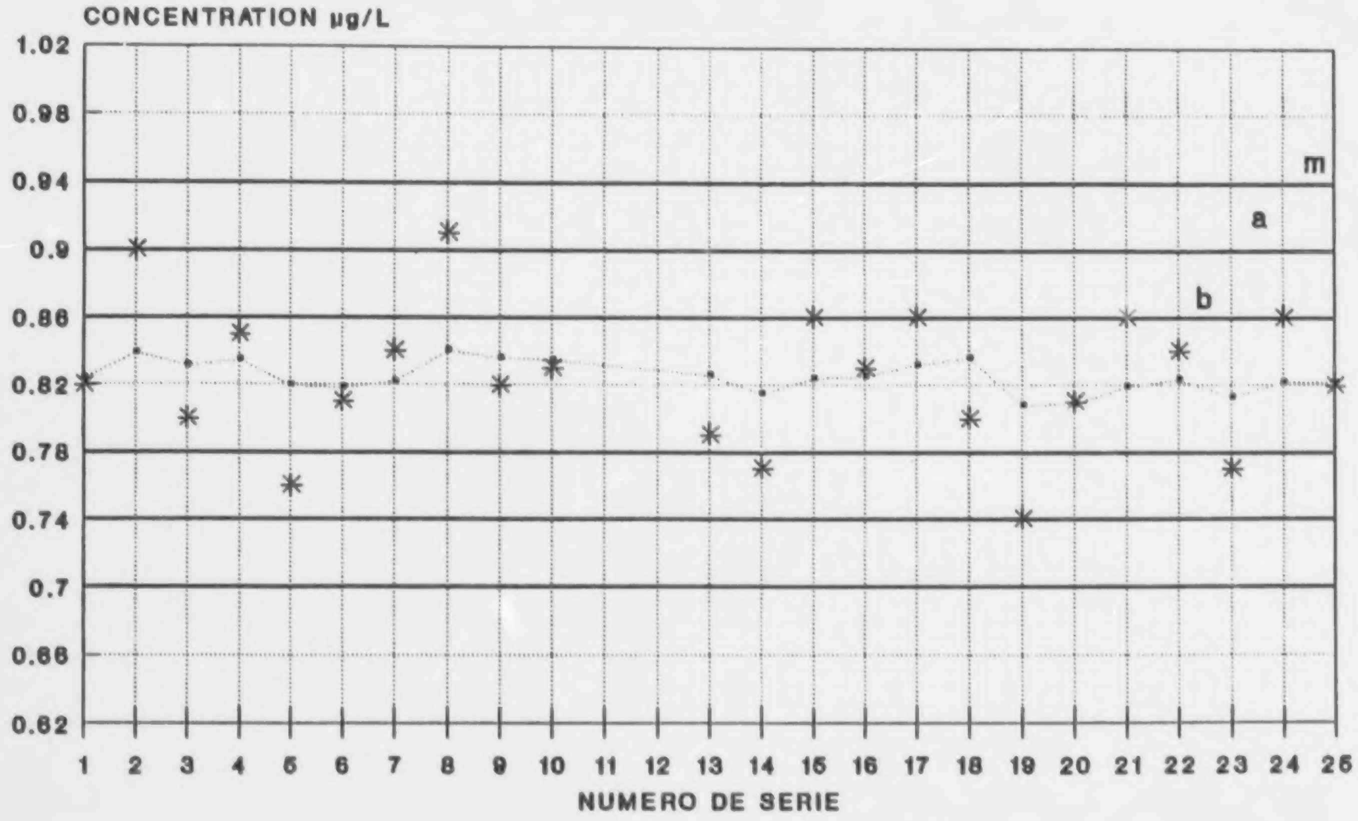
DIAGRAMME DE CONTROLE C/P
BPC #138 DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.73
SIGMA=.04 C.V.=5.40%

DIAGRAMME DE CONTROLE C/P

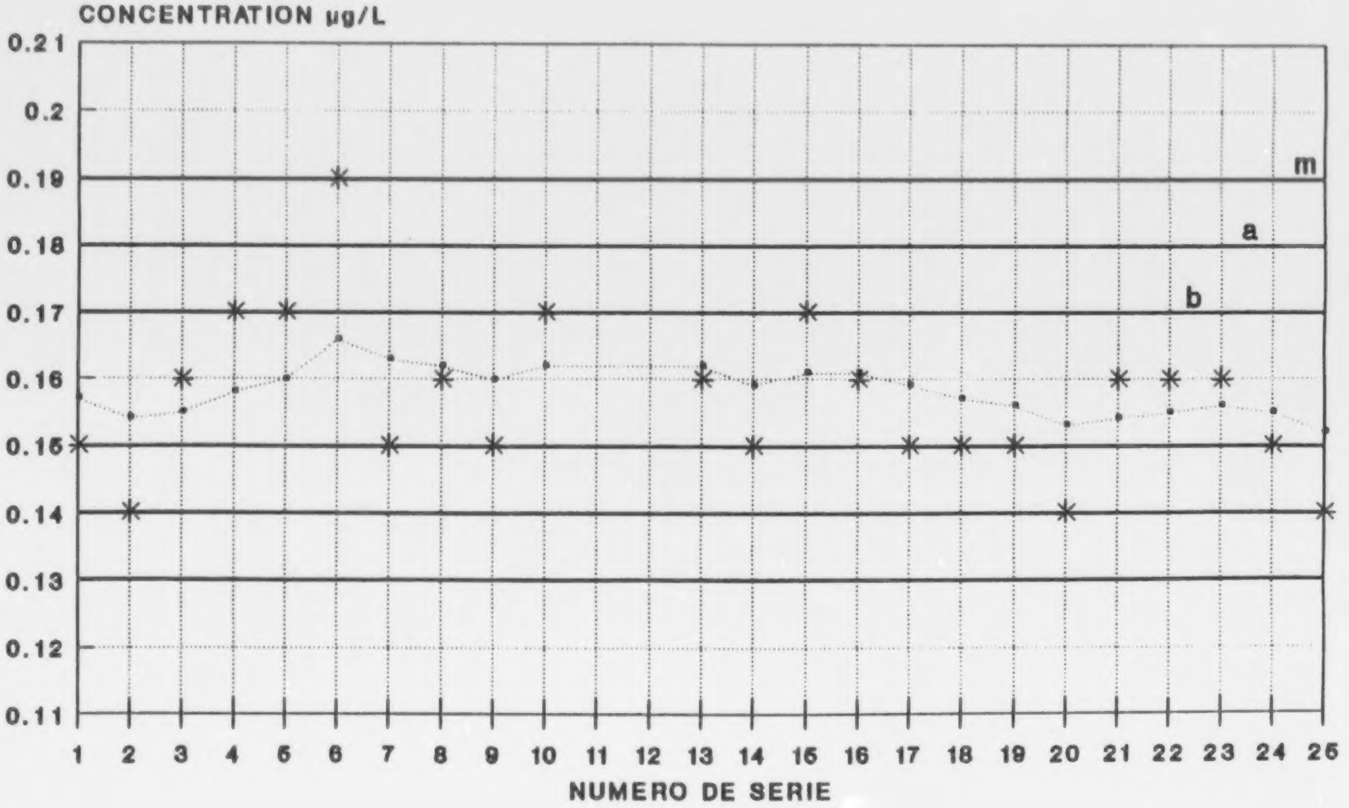
BPC #153 DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.82
SIGMA=.04 C.V.=5.13%

DIAGRAMME DE CONTROLE C/P

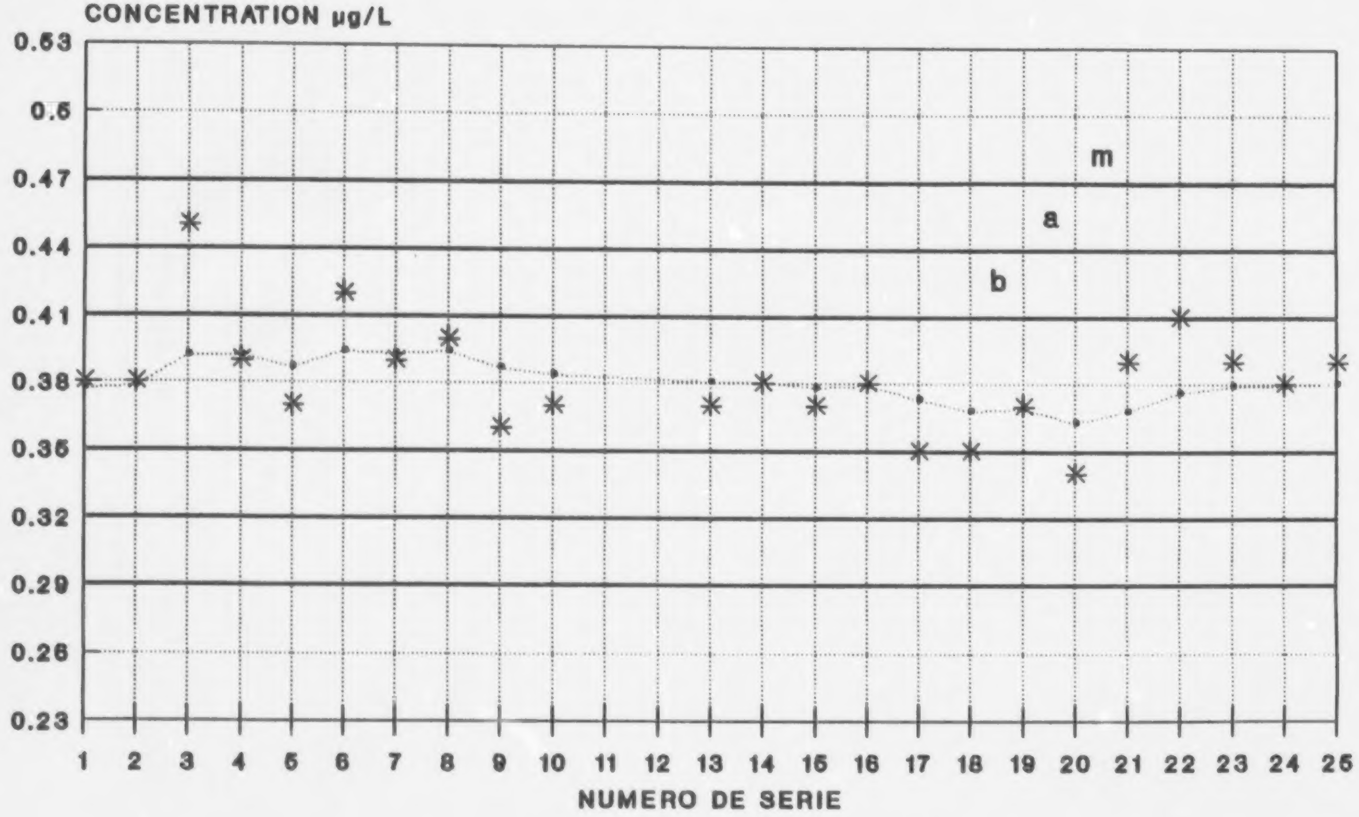
BPC #156 DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.16
SIGMA=.01 C.V.=7.55%

DIAGRAMME DE CONTROLE C/P

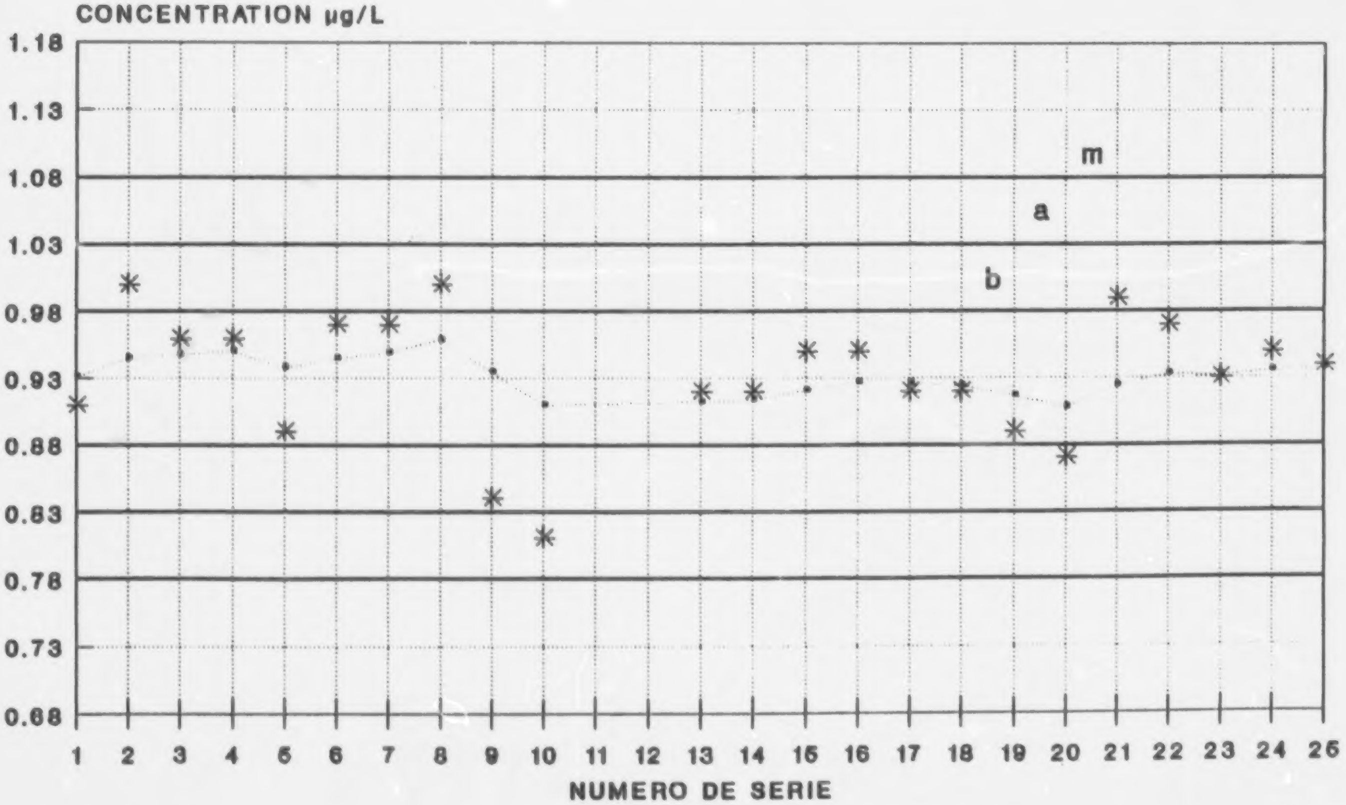
BPC #170 DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.38
SIGMA=.03 C.V.=7.74%

DIAGRAMME DE CONTROLE C/P

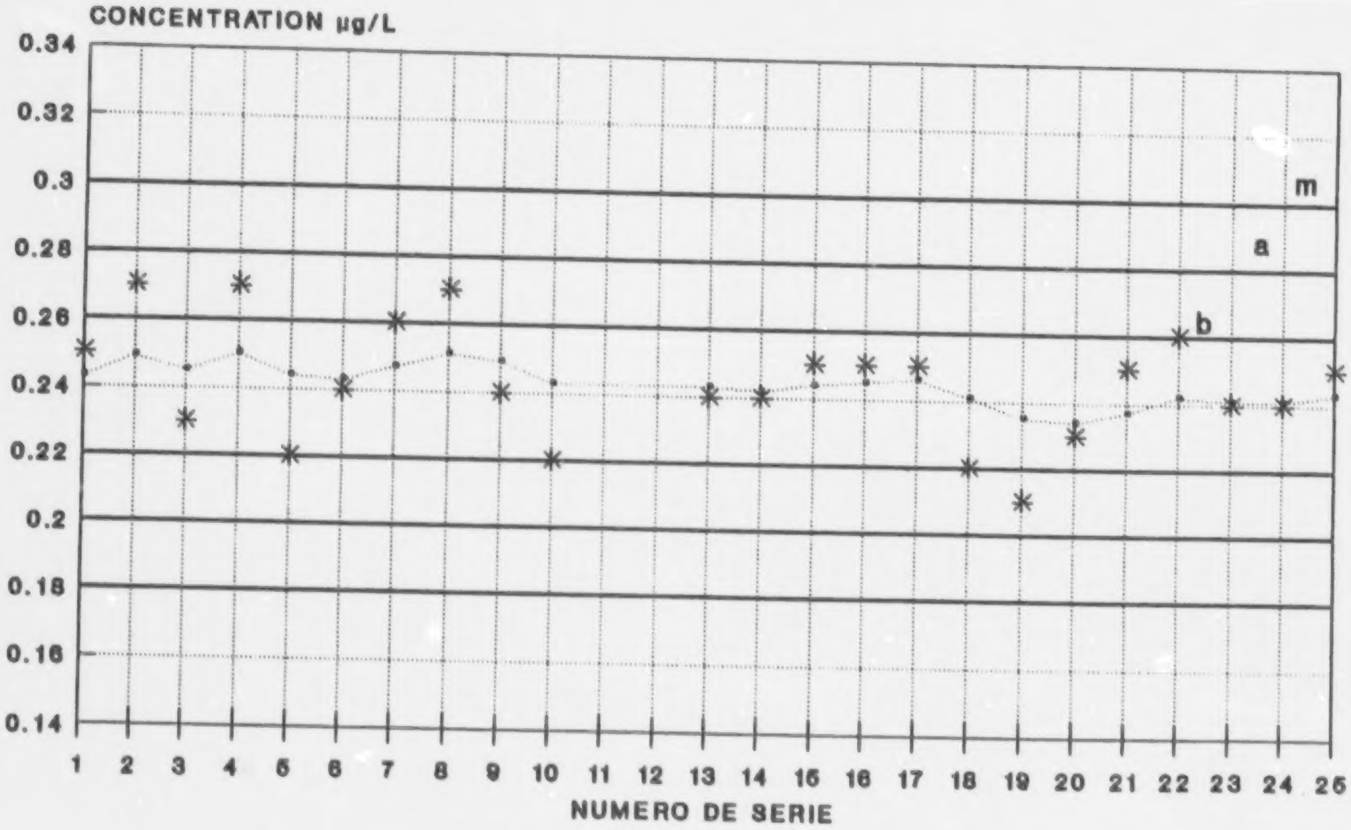
BPC #180 DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.93
SIGMA=.05 C.V.=5.18

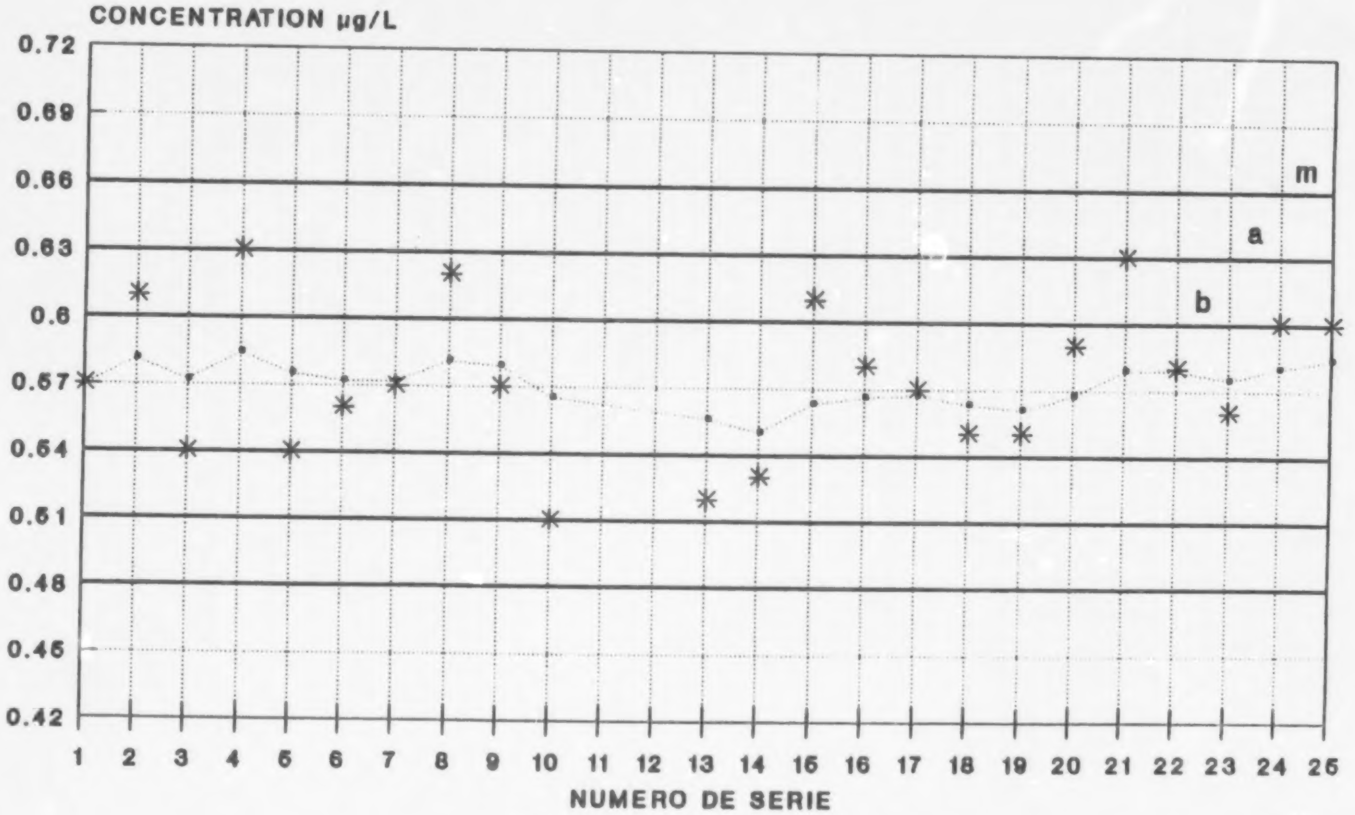
DIAGRAMME DE CONTROLE C/P

BPC #183 DANS LE PLASMA
CONTROLE NBS 1/10, 921119



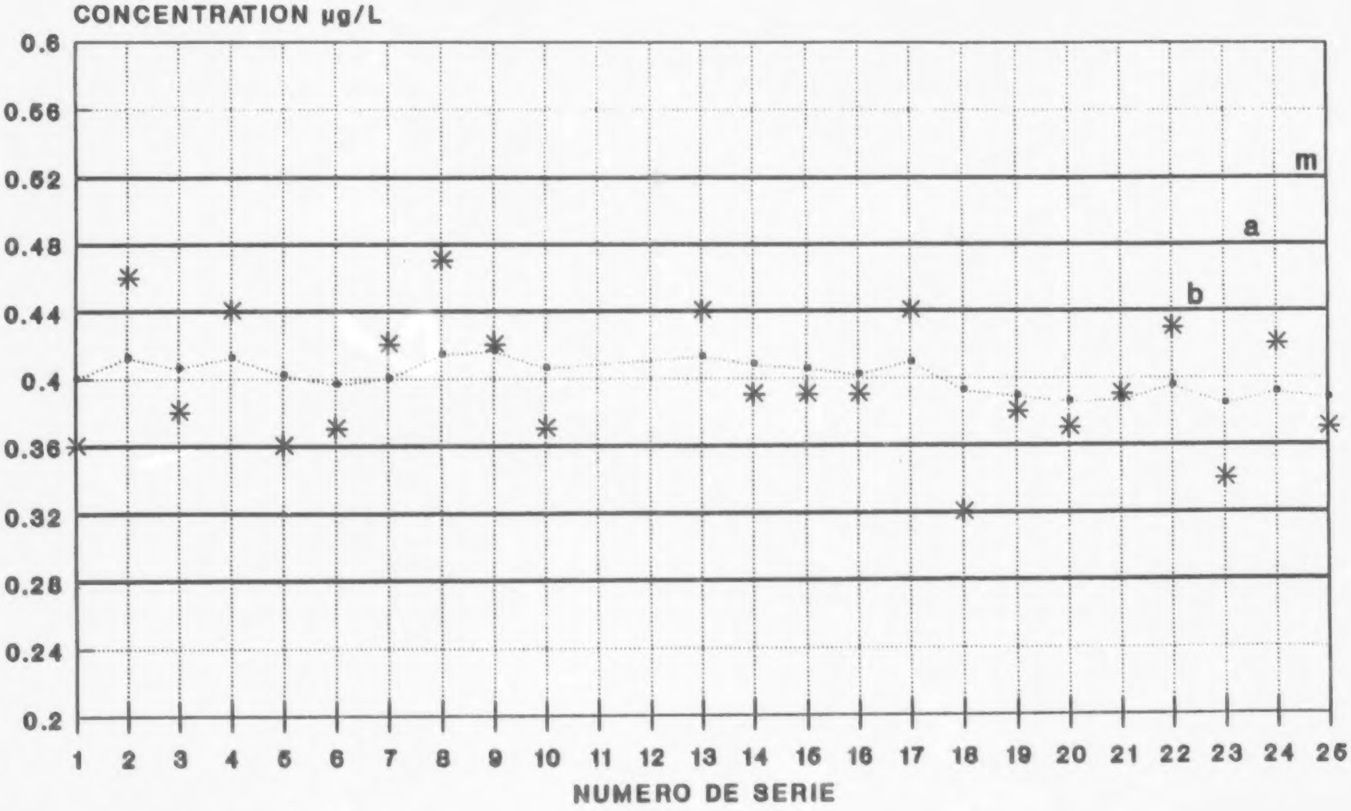
PERIODE (23/11/92-01/02/93)
MOYENNE =.24
SIGMA=.02 C.V.=6.85%

DIAGRAMME DE CONTROLE C/P
BPC #187 DANS LE PLASMA
CONTROLE NBS 1/10, 921119



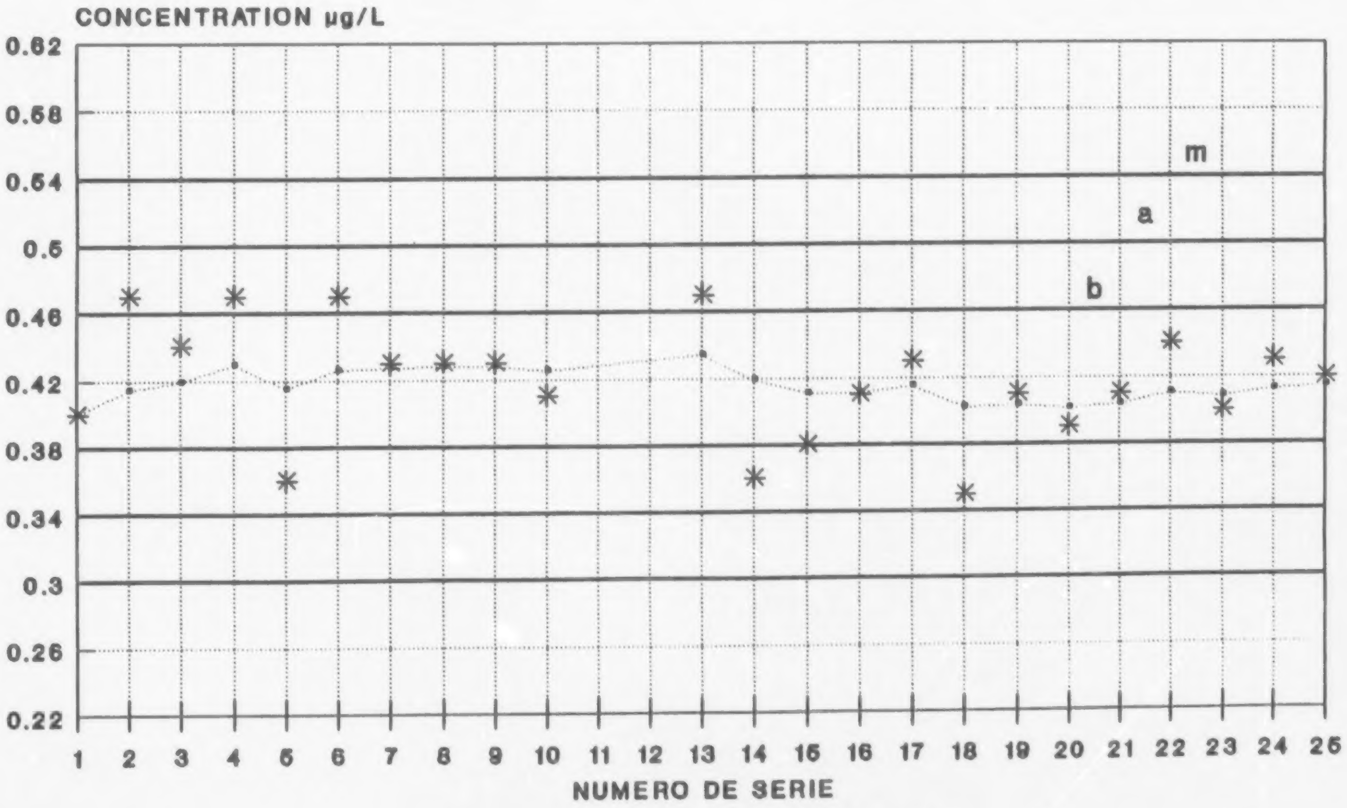
PERIODE (23/11/92-01/02/93)
MOYENNE =.57
SIGMA=.03 C.V.=5.94%

DIAGRAMME DE CONTROLE C/P
B-BHC 0.4ug/L DANS LE PLASMA
CONTROLE NBS 1/10, 921119



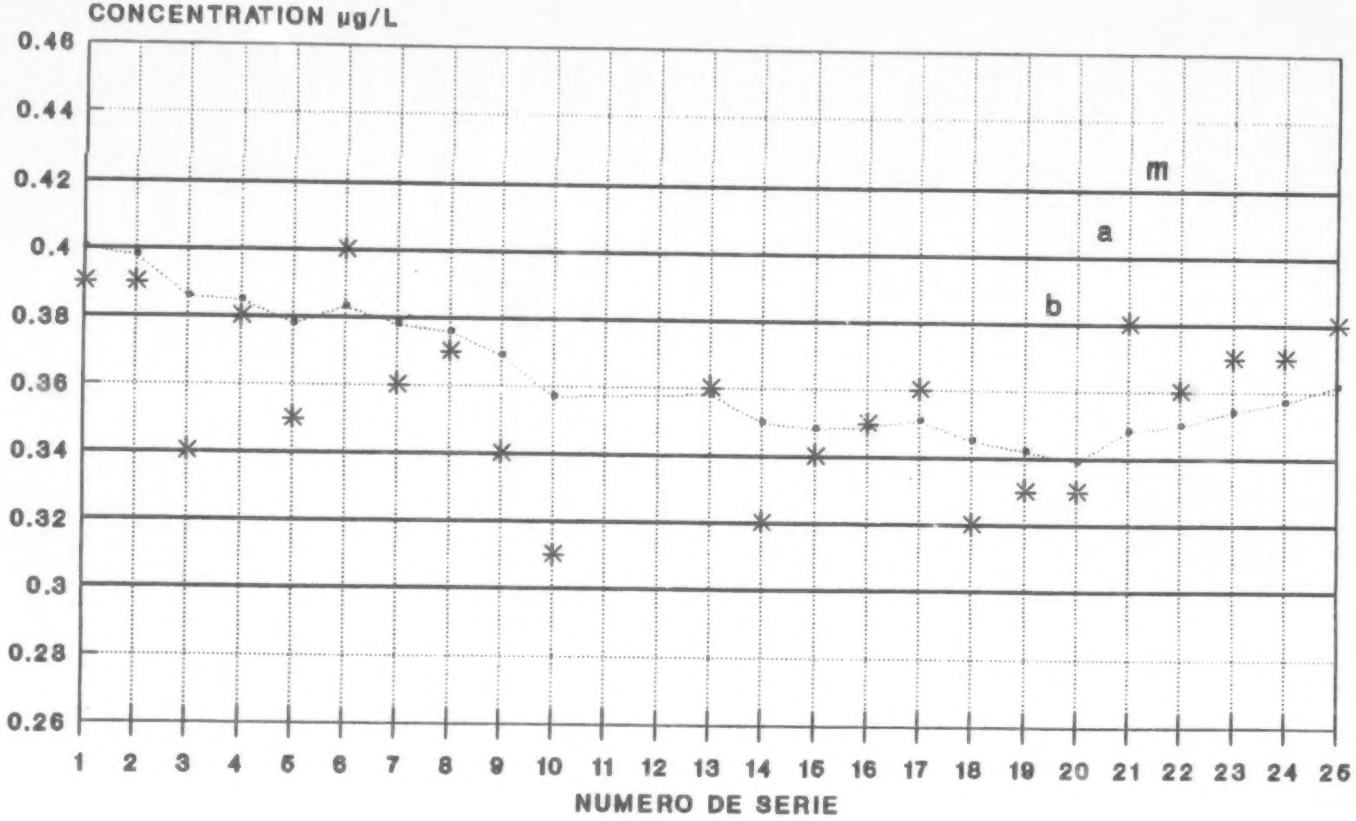
PERIODE (23/11/92-01/02/93)
MOYENNE =.40
SIGMA=.04 C.V.=9.79%

DIAGRAMME DE CONTROLE C/P
ALPHACHLORDANE 0.4ug/L DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.42
SIGMA=.04 C.V.=8.38%

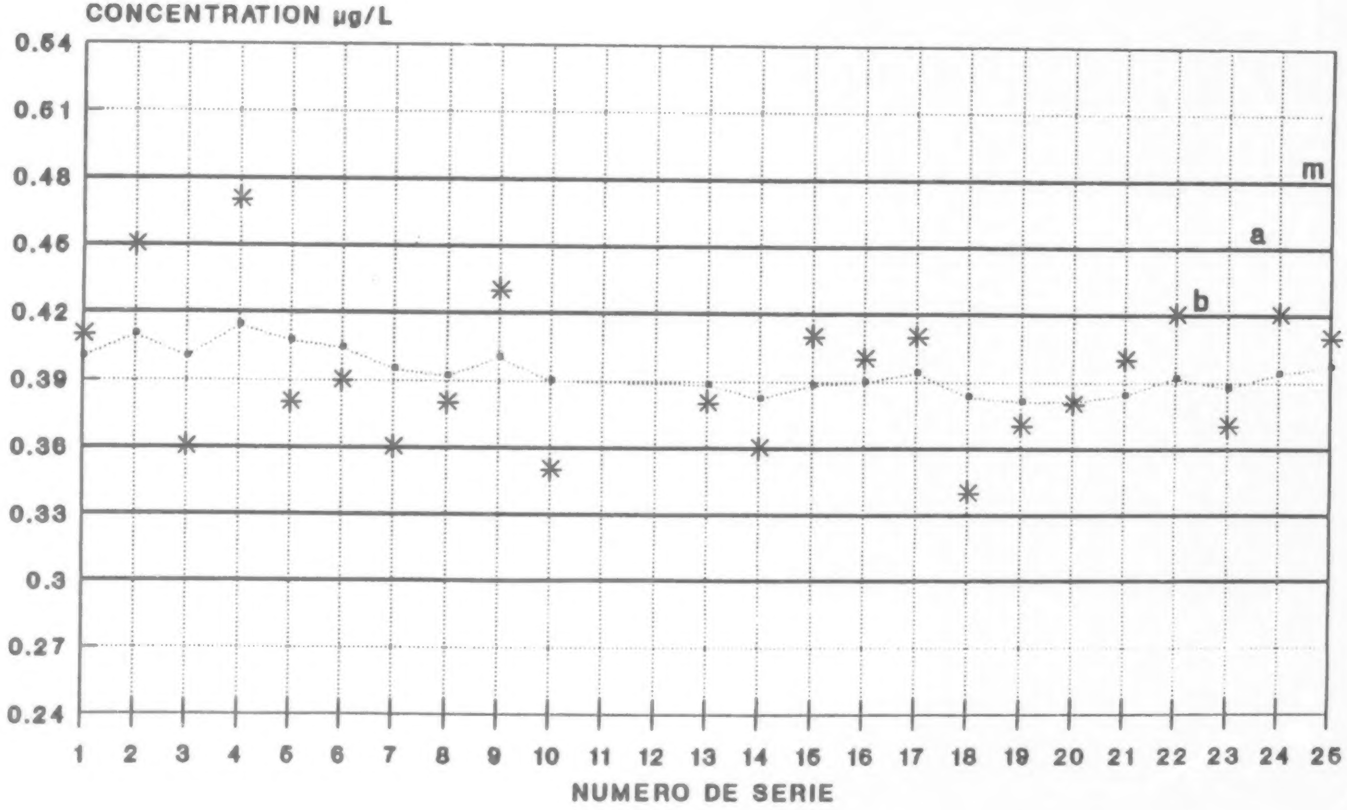
DIAGRAMME DE CONTROLE C/P
GAMMACHLORDANE DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.36
SIGMA=.02 C.V.=6.95%

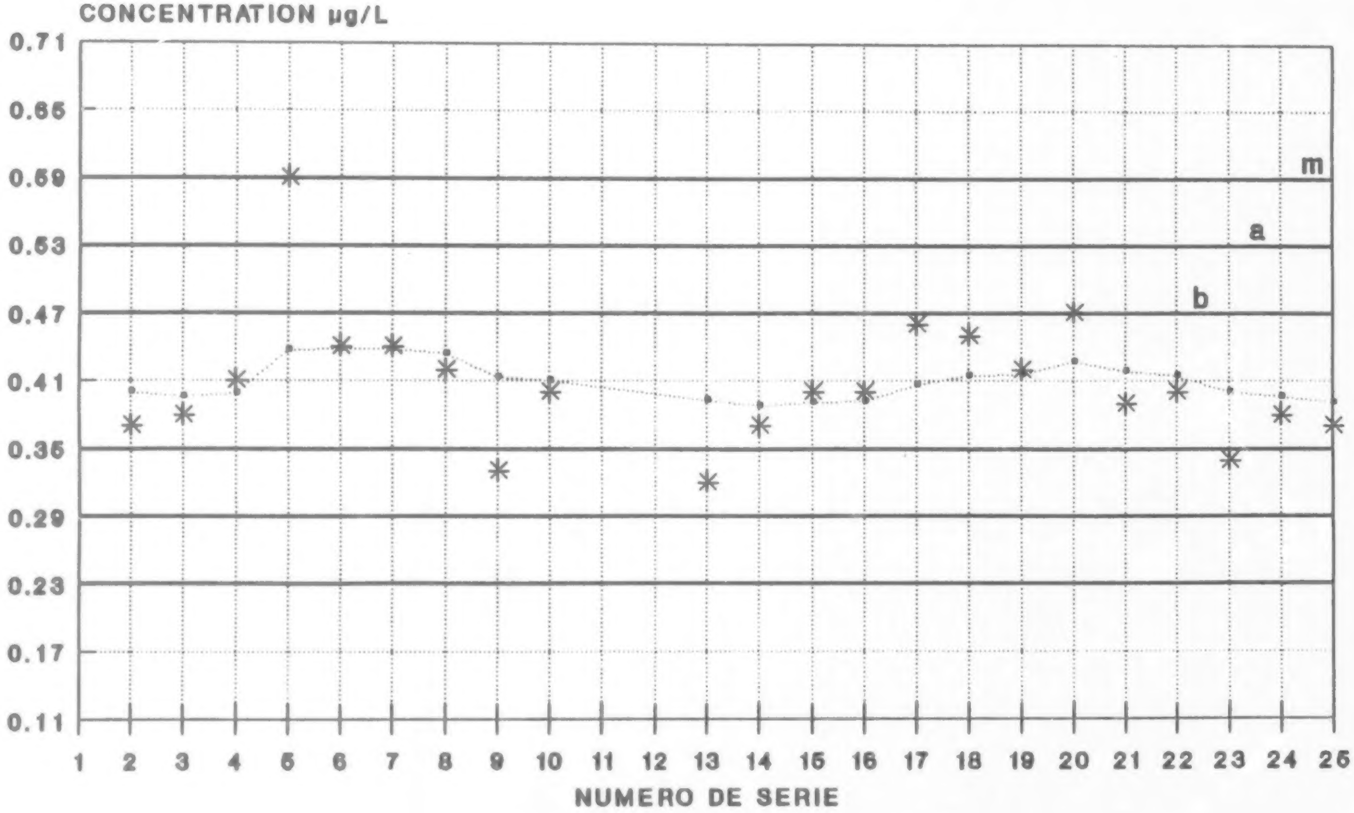
DIAGRAMME DE CONTROLE C/P

pp'DDE 0.4ug/L DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.39
SIGMA=.03 C.V.=8.20%

DIAGRAMME DE CONTROLE C/P
DIELDRIN 0.4ug/L DANS LE PLASMA
CONTROLE NBS 1/10, 921119

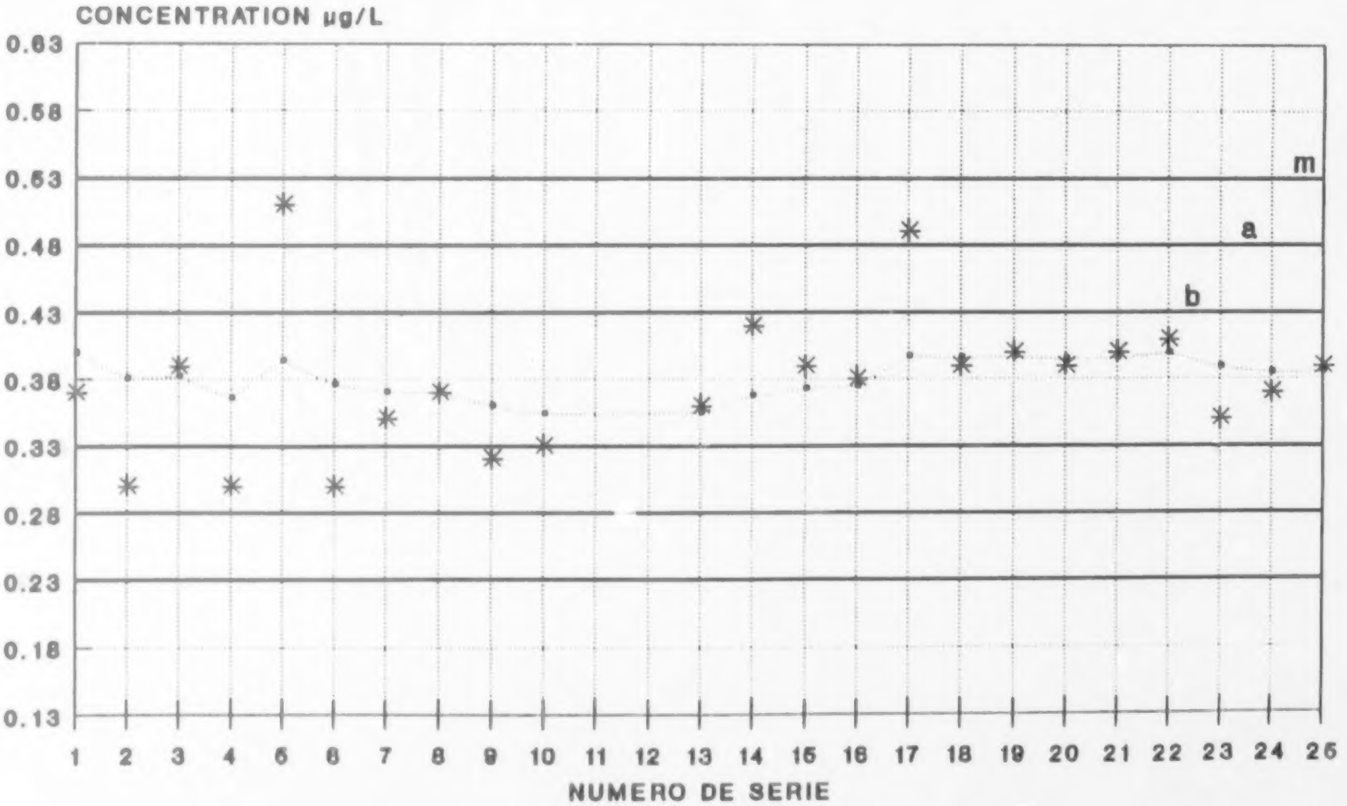


PERIODE (23/11/92-01/02/93)
MOYENNE =.41
SIGMA=.06 C.V.=13.79%

DIAGRAMME DE CONTROLE C/P

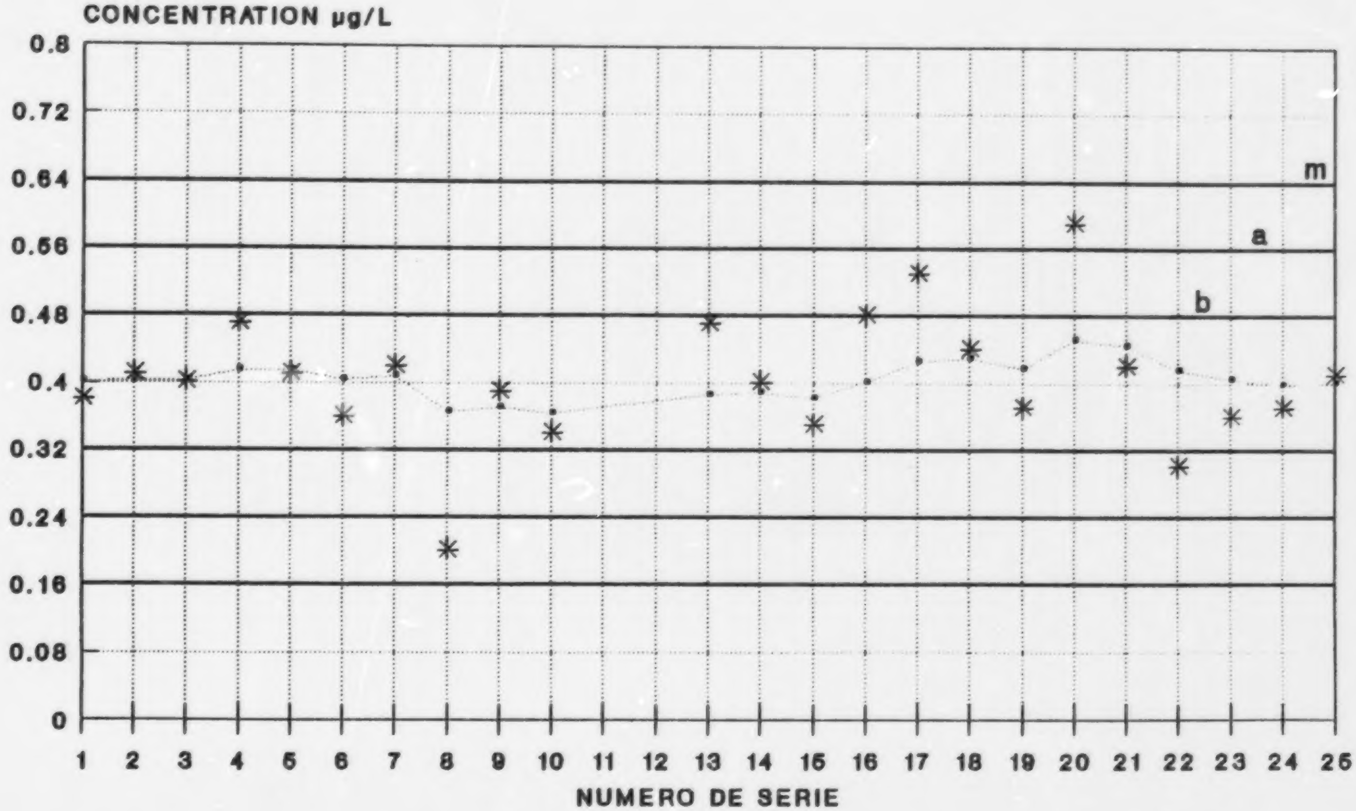
HEPTACHLOREPOXIDE 0.4ug/L DANS LE PLASMA

CONTROLE NBS 1/10, 921119



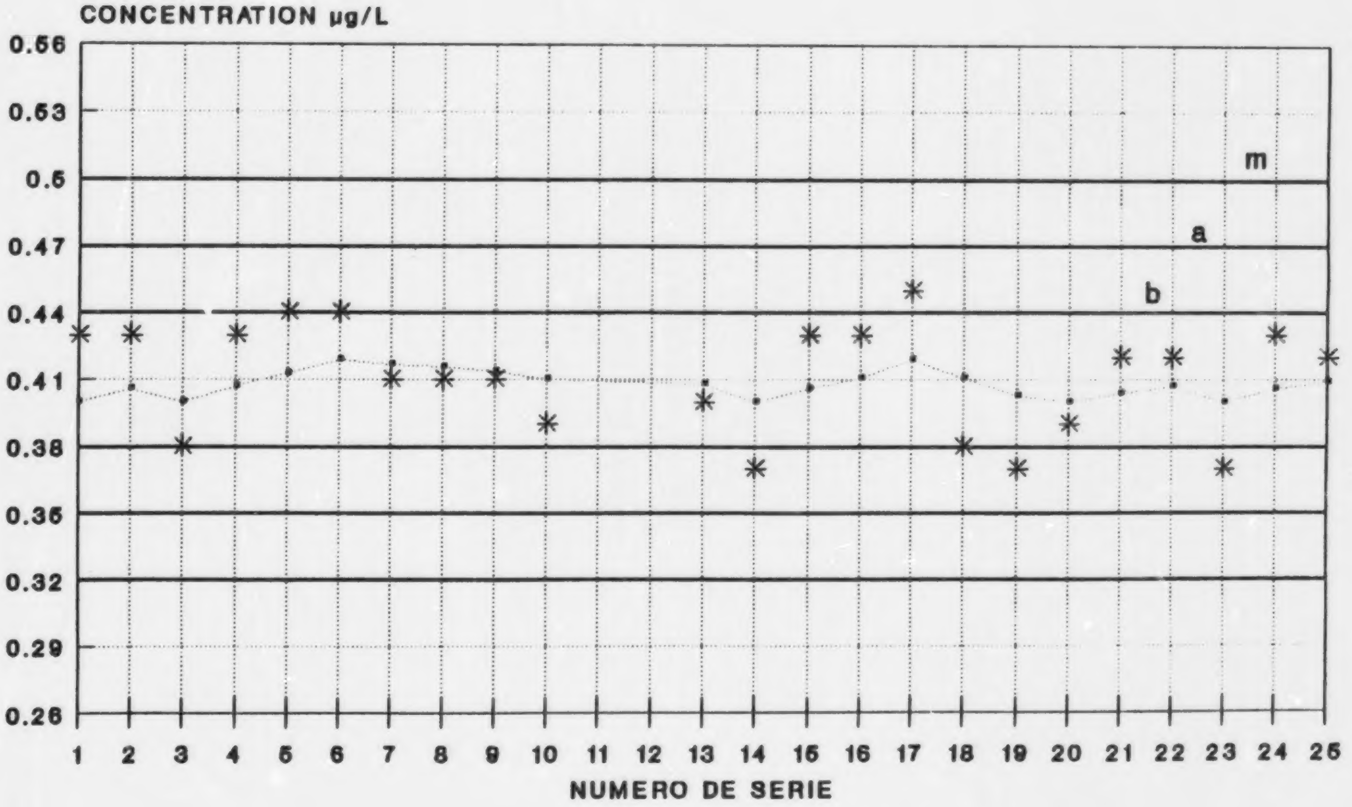
PERIODE (23/11/92-01/02/93)
MOYENNE =.38
SIGMA=.06 C.V.=15.22%

DIAGRAMME DE CONTROLE C/P
HEXACHLOROBENZENE 0.4ug/L DANS LE PLASMA
CONTROLE NBS 1/10, 921119



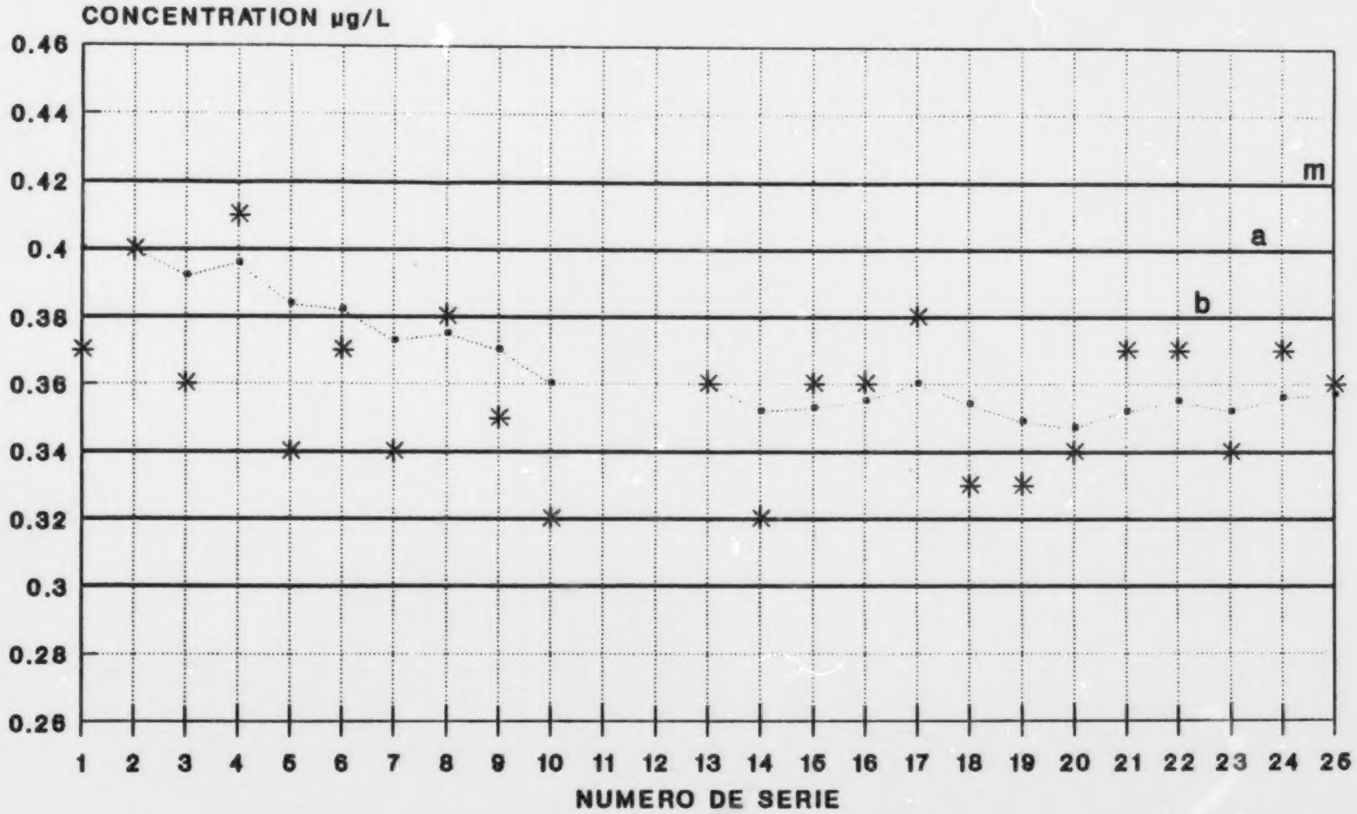
PERIODE (23/11/92-01/02/93)
MOYENNE =.40
SIGMA=.08 C.V.=19.31%

DIAGRAMME DE CONTROLE C/P
MIREX 0.4ug/L DANS LE PLASMA
CONTROLE NBS 1/10, 921119



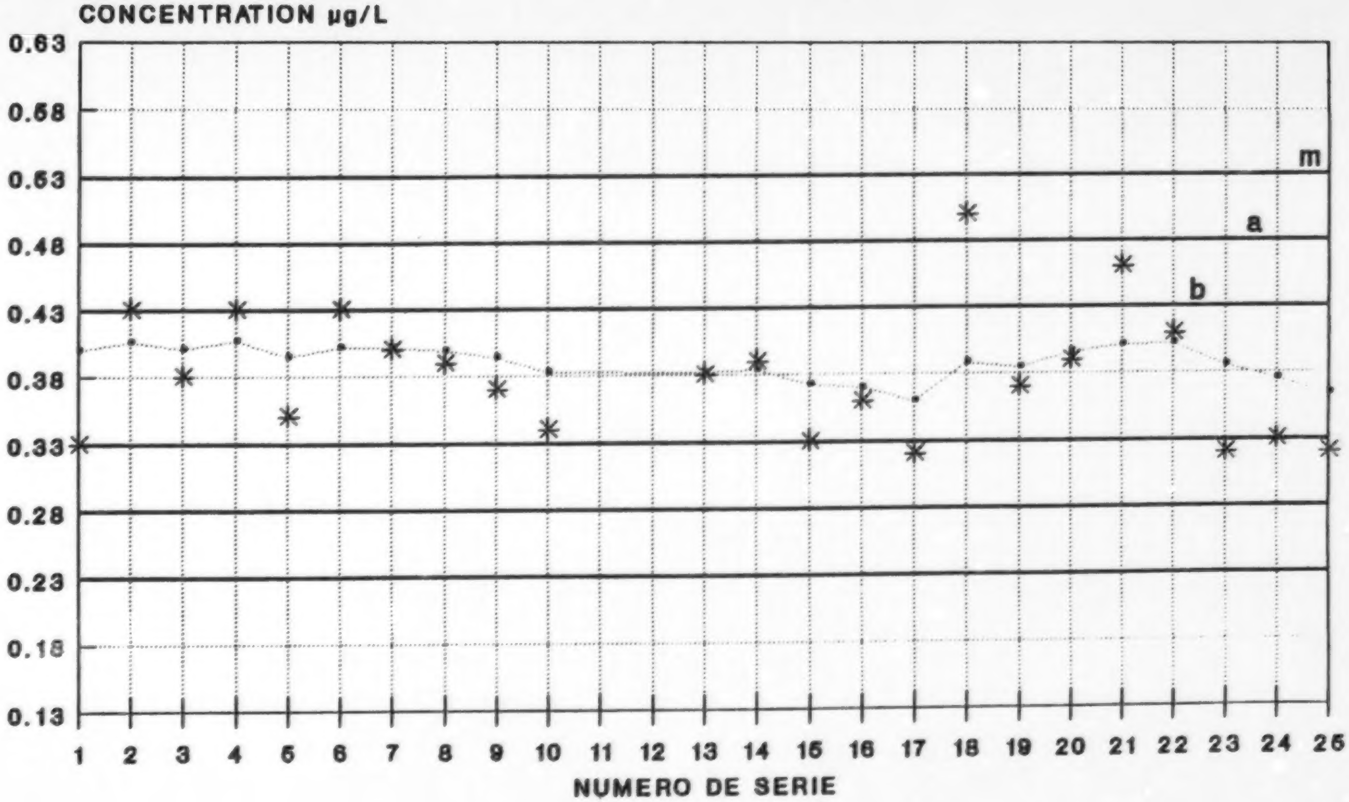
PERIODE (23/11/92-01/02/93)
MOYENNE =.41
SIGMA=.03 C.V.=6.82%

DIAGRAMME DE CONTROLE C/P
TRANS NONACHLOR 0.4ug/L DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.36
SIGMA=.02 C.V.=6.52%

DIAGRAMME DE CONTROLE C/P
OXYCHLORDANE 0.4ug/L DANS LE PLASMA
CONTROLE NBS 1/10, 921119



PERIODE (23/11/92-01/02/93)
MOYENNE =.38
SIGMA=.05 C.V.=12.72%

B. Metals Analysis in Blood and Hair

Reference Materials

1. Blood

The reference materials for blood were supplied by the Occupational Health Unit, Medical Services Branch. They consisted of Control Blood for Metals 1 (Behring) of known values for lead, cadmium, mercury, and chromium.

2. Hair

There is no known reference material for metals in hair. Instead, hair of sufficient length and quantity was obtained from a female volunteer with very long hair.

Preparation of Quality Control Check Samples

1. Blood

Seven Control Blood samples were reconstituted with distilled water. The reconstituted blood samples were then transferred to metal free, royal blue top (BD 6527) vacutainers, identical to those used to collect blood samples from the study participants.

2. Hair

The hair, which was collected according to the procedures described in section I. C., was carefully divided into five smaller samples. These samples were placed into polyethylene bags identical to those used to collect hair samples from the study participants.

Labelling and Handling of Quality Control Check Samples

Dummy identification numbers and sampling dates were predetermined for all analytical QC check samples (blood and hair). All vacutainers and polyethylene bags containing these samples were labelled in the same fashion as samples collected from the study participants. Each week, one hair and blood sample was included with the regular shipment of samples to the laboratory. A BLOOD SPECIMEN REPORT FORM and a HAIR SPECIMEN REPORT FORM was filled out for each blood and hair sample and accompanied it to its destination.

Results

Metals in Blood: Quality Control Check Samples (n = 7)

Contaminant		Mean	Standard Deviation	Coefficient of Variation (%)
Lead (µg/100 mL)		35.66	1.47	4.12
Cadmium (µg/L)		5.06	0.18	3.58
Mercury *	Inorganic (ppb)	10.14	0.69	6.80
	Total (ppb)	10.00	0.82	8.16

* All mercury in these control blood samples was comprised of inorganic mercury. Therefore the inorganic and total mercury contents measured in the blood were identical.

The assigned values of the control blood samples, as determined by laboratories of the German Society for Industrial Medicine, are presented below.

Contaminant	**Assigned Value**	**Confidence Range**
Lead (µg/100 mL)	41.3	37.7 - 44.9
Cadmium (µg/L)	5.2	4.3 - 6.1
Mercury (Total, ppb)	10.4	8.5 - 12.3

Note: The date of expiry for the control blood samples was December 31, 1991. These samples were analyzed in February 1993. These control blood samples were used even though they were beyond the expiry date because they were the only known control samples with lead, cadmium and mercury. No other supplier could be found. In addition, the supplier (Behring) had discontinued supplying this type of control blood. The samples used in this study had been purchased previously for other purposes and stored in a refrigerator. It was felt that the metal levels would have remained sufficiently stable to use even after the expiry date.

APPENDIX 1 Pre-Study Laboratory Audit: PCBs and Organochlorine Pesticides in Plasma

Principles of the Audit

Prior to the initiation of the study, the laboratory chosen to perform the analysis of samples for the determination of PCBs and organochlorine pesticides was audited by study personnel. The audit consisted of submitting human plasma samples spiked with known levels of reference material to the laboratory for analysis. The levels of contaminants and the spiking scheme were not known by the analytical laboratory. Results were evaluated in order to assess the precision of the laboratory's analytical method.

Standard Reference Material

All standard reference materials were provided by the analytical laboratory and consisted of the following:

Solutions of PCB congeners 138, 153, 180, 183 and 187 at 1 mg/L in methanol;

Solutions of mirex, dieldrin, hexachlorobenzene, and endrin at 1 mg/L in methanol;

Solution of DDE at 10 mg/L in methanol.

Blood Source

Approximately 200 mL of fresh human plasma was provided by Le Centre de Toxicologie du Québec. The plasma was previously analyzed by the supplier to ensure that its levels of contaminants were low.

Spike Preparation

The spiked samples were prepared by the Organic Chemistry Section, Monitoring and Criteria Division of Health and Welfare Canada.

Twelve (12) vials with teflon lined caps were used for spiking, each with 10 compounds to be analysed. One vial was used as an unspiked sample. In each of the 11 remaining vials, mixtures of all 10 different compounds were prepared in human plasma. Concentrations at 0 x and 1 x the analytical limits of quantification, and a concentration equivalent to average values in human populations (based on current literature) were used for each of the 10 target compounds and 4 replicates at each concentration were prepared. The plasma spiking scheme followed is outlined in the table below. The contaminant concentrations were randomly assigned to each tube. A duplicate sample, prepared concurrently, was retained in case of future need.

Each vial was labelled with the vial number, the date of preparation of the spiked sample, and the initial of the scientist who prepared the spiked plasma samples. These samples were then shipped to the analytical laboratory in a styrofoam shipping box with ice packs to keep the samples cold.

PLASMA SPIKING SCHEME

Vial No.	1	2	3	4	5	6
Compound	P180: 0	P180: 0.05	P180: 1.5	P180: 0	P180: 0	P180: 1.5
(µg/L)	P138: 0	P138: 0	P138: 0.05	P138: 1.5	P138: 0	P138: 1.5
	DDE: 0.05	DDE: 10.0	DDE: 0	DDE: 10.0	DDE: 0	DDE: 0
	DIE: 0.05	DIE: 0	DIE: 0.2	DIE: 0.2	DIE: 0	DIE: 0.05
	MIR: 0.05	MIR: 0	MIR: 0	MIR: 0.2	MIR: 0	MIR: 0.2
	P153: 0	P153: 0	P153: 0	P153: 2.0	P153: 0	P153: 0.05
	P187: 0.05	P187: 1.0	P187: 0	P187: 0.05	P187: 0	P187: 0.05
	END: 0.1	END: 0.05	END: 0.1	END: 0.05	END: 0	END: 0.1
	HCB: 0	HCB: 0.3	HCB: 0.05	HCB: 0.3	HCB: 0	HCB: 0.05
	P183: 0.05	P183: 0.3	P183: 0.3	P183: 0.05	P183: 0	P183: 0.05

Vial No.	7	8	9	10	11	12
Compound	P180: 1.5	P180: 0.05	P180: 0.05	P180: 0.05	P180: 0	P180: 1.5
(µg/L)	P138: 0.05	P138: 0.05	P138: 1.5	P138: 0	P138: 1.5	P138: 0.05
	DDE: 0.05	DDE: 0.05	DDE: 10.0	DDE: 10.0	DDE: 0.05	DDE: 0
	DIE: 0.05	DIE: 0	DIE: 0	DIE: 0.2	DIE: 0.05	DIE: 0.2
	MIR: 0.2	MIR: 0.05	MIR: 0	MIR: 0.05	MIR: 0.2	MIR: 0.05
	P153: 0.05	P153: 0.05	P153: 2.0	P153: 2.0	P153: 2.0	P153: 0.05
	P187: 1.0	P187: 0.05	P187: 1.0	P187: 1.0	P187: 0	P187: 0
	END: 0	END: 0	END: 0.05	END: 0	END: 0.1	END: 0.05
	HCB: 0	HCB: 0.05	HCB: 0.3	HCB: 0.05	HCB: 0.3	HCB: 0
	P183: 0.05	P183: 0	P183: 0.3	P183: 0	P183: 0.3	P183: 0

P138: PCB congener 138
P153: PCB congener 153
P180: PCB congener 180
P183: PCB congener 183
P187: PCB congener 187
DDE: DDE
DIE: Dieldrin
END: Endrin
HCB: Hexachlorobenzene
MIR: Mirex

Analytical Laboratory

The analytical laboratory audited was the Centre de Toxicologie du Québec, Centre Hospitalier de l'Université Laval, Ste. Foy, Québec.

Results

Pre-Study Laboratory Audit: PCBs and Organochlorine Pesticides in Plasma

Compound	Spiked Level (µg/L)	Mean Measured [a] (µg/L)	Corrected Mean [b] (µg/L)	Standard Deviation	Coefficient of Variation (%)
DDE	0.00	0.1550 (4,4)	0.0000	0.0129	.
	0.05	0.2000 (4,4)	0.0450	0.0082	18.144
	10.00	11.7500 (4,4)	11.595	1.2583	10.852
Dieldrin	0.00	0.0075 (0,0)	0.0000	0.0000	.
	0.05	0.0650 (4,4)	0.0575	0.0129	22.452
	0.20	0.2000 (4,4)	0.1925	0.0216	11.222
Endrin	0.00	0.0075 (0,0)	0.0000	0.0000	.
	0.05	0.0725 (4,4)	0.0650	0.1500	23.077
	0.10	0.1325 (4,4)	0.1250	0.0275	22.030
Hexachlorobenzene	0.00	0.0075 (0,0)	0.0000	0.0000	.
	0.05	0.0550 (4,4)	0.0475	0.0058	12.155
	0.30	0.2900 (4,4)	0.2825	0.0116	4.087
Mirex	0.00	0.0075 (0,0)	0.0000	0.0000	.
	0.05	0.0131 (1,3)	0.0056	0.0113	200.000
	0.20	0.1550 (4,4)	0.1475	0.0129	8.753
PCB 138	0.00	0.0250 (4,0)	0.0000	0.0058	.
	0.05	0.0875 (4,4)	0.0625	0.0050	8.000
	1.50	1.6250 (4,4)	1.6000	0.1708	10.674
PCB 153	0.00	0.0400 (4,0)	0.0000	0.0000	.
	0.05	0.0975 (4,4)	0.0575	0.0206	35.853
	2.00	1.7500 (4,4)	1.7100	0.1291	7.550
PCB 180	0.00	0.0300 (4,0)	0.0000	0.0082	.
	0.05	0.0775 (4,4)	0.0475	0.0050	10.526
	1.50	1.1250 (4,4)	1.3950	0.0957	6.863
PCB 183	0.00	0.0075 (0,0)	0.0000	0.0000	.
	0.05	0.0350 (4,0)	0.0275	0.0058	20.995
	0.30	0.2725 (4,4)	0.2650	0.0150	5.660
PCB 187	0.00	0.0075 (0,0)	0.0000	0.0000	.
	0.05	0.0575 (4,4)	0.0500	0.0050	10.00
	1.00	0.8900 (4,4)	0.8825	0.0497	5.628

Numbers in parentheses: The first value indicates the number of samples above L.O.D.
The second value indicates the number of samples above L.O.Q.

[a] In cases where the measured values were below the detection limit, the means were calculated using values at one-half of the detection limit. In cases where measured values were below the level of quantification, the reported value was used.

[b] Corrected Mean = Mean Measured - Mean Measured at 0.00 Spiked Level

APPENDIX 2 Laboratory Quality Control for PCBs and Organochlorine Pesticides

Analytical Precision: Calculated from Standard Samples (n = 25)

Compounds	Spiking Level or Mean Concentration (μg/L)	Standard Deviation	Coefficient of variation (%)
Aldrin	0.4	0.042	10.4
α-chlordane	0.4	0.034	8.4
γ-chlordane	0.4	0.028	6.9
p,p'-DDE	0.4	0.033	8.2
Dieldrin	0.4	0.055	13.8
β-HCH	0.4	0.039	9.8
Heptachlor Epoxide	0.4	0.060	15.0
Hexachlorobenzene	0.4	0.076	19.0
Mirex	0.4	0.027	6.8
Cis-nonachlor	0.4	0.038	9.5
Trans-nonachlor	0.4	0.026	6.5
Oxychlordane	0.4	0.051	12.7
PCB (total)	8.0	0.43	5.4
PCB 101	0.31	0.030	9.6
PCB 105	0.24	0.023	9.5
PCB 118	0.91	0.076	8.3
PCB 138	0.73	0.039	5.4
PCB 153	0.82	0.042	5.1
PCB 156	0.16	0.012	7.6
PCB 170	0.38	0.029	7.7
PCB 180	0.93	0.048	5.2
PCB 183	0.24	0.016	6.9
PCB 187	0.57	0.034	5.9

Percent Recovery: Standard Samples

Compounds	Spiking Level or Mean Concentration (μg/L)	Mean Percent Recovery (n = 23)
α-chlordane	0.4	103
γ-chlordane	0.4	90
p,p'-DDE	0.4	98
Dieldrin	0.4	103
β-HCH	0.4	100
Heptachlor Epoxide	0.4	95
Hexachlorobenzene	0.4	100
Mirex	0.4	103
Trans-nonachlor	0.4	90
Oxychlordane	0.4	95
PCBs (Total and Congeners)	0.5	> 95%

APPENDIX 3 Disposition of Biological Samples

All analyses of biological samples collected in this study have been approved by the Health Protection Branch Human Ethics Committee and have been performed with the signed consent of the study participants. Further analysis of any of these biological samples will require additional approval by the Ethics Committee. In addition, all study participants whose samples have been identified for further analysis must be contacted, by letter, in order to obtain their signed consent.

Appendix 7
Interpretation of biological sample results for participants and physicians

Criteria and text sequences

Contents

Serum lipids . . . . App. 7-2
PCBs . . . . App. 7-3
Other organochlorines . . . . App. 7-5
Mercury . . . . App. 7-6
Lead . . . . App. 7-7
Cadmium . . . . App. 7-8
Liver activity measurements . . . . App. 7-9
Urinary porphyrins . . . . App. 7-10
d-Glucaric acid . . . . App. 7-11
Thyroid hormones . . . . App. 7-12

Serum lipids

The following serum lipids were measured by the Ottawa Civic Hospital laboratory from a non-fasted sample:

Total cholesterol (mmol/L)
Triglycerides (mmol/L)
Free cholesterol (mmol/L)
Phospholipids (mmol/L)

Reference ranges has been established by the Civic Hospital for total cholesterol and triglycerides (for fasted samples)[1]:

	Reference range (fasted sample)
Total cholesterol (mmol/L)	≤5.2 mmol/L
Triglycerides	0.55-1.70 mmol/L

---

[1] Handbook of biochemical tests and reference values. 1992. Civic Hospital, Biochemistry Laboratory.

In discussion with Dr. S. Perkins (Ottawa Civic Hospital) it was decided that participants (and physicians) would be notified immediately by letter if the total cholesterol value exceeded 7 mmol/L or the triglycerides exceeded 9 mmol/L. The letters recommended retesting 'if the finding was new and the clinical situation warranted'. These results were provided again in the July 7 1993 mail-out of results, with reference to the earlier letters.

Serum Lipids

Criteria	Text used in Summary Sheet to Participants	Text used in Covering Letter to Physician
Total cholesterol ≤ 5.2 mmol/L and Triglycerides ≤ 4 mmol/L	'Your cholesterol level was within the range recommended for Canadian adults.'	n/a
Total cholesterol between 5.2-7.0 mmol/L or triglyceride ≥4 mmol/L	'Your cholesterol and/or triglyceride level was slightly higher than the recommended level. We do not feel this requires immediate attention but we suggest you discuss this result with your doctor at your next regular visit.'	'The cholesterol and/or triglyceride value was slightly elevated, but this result was from a non-fasted sample. We recommend retesting if this finding is new and the clinical situation warrants.'
Total cholesterol ≥7 mmol/L or triglyceride ≥9 mmol/L (letters would have been sent earlier to participant and physician)	'Your cholesterol or triglyceride level was above the recommended level. We contacted you earlier this year about this result.'	'The cholesterol or triglyceride value was above the recommended level, but this result was from a non-fasted sample. This result was provided earlier this year.'

PCBs (measured as Arochlor 1260, μg/L)

The following 'guidelines' were developed within the Health Protection Branch in 1986[1]:

	Women of reproductive age	Men and post-menopausal women
Tolerable	<5 μg/L	<20 μg/L
Concern	5-<100 μg/L	20-<100 μg/L
Action	≥100 μg/L	≥100 μg/L

PCBs		
Criteria	**Text used in Summary Sheet to Participants**	**Text used in Covering Letter to Physician**
Women in reproductive age group - <5 μg/L **Males and post-menopausal women - <20 μg/L**	'The levels of PCBs and the other environmental contaminants such as DDT, in your blood, were in the range normally found in the Canadian general population. Many environmental contaminants such as PCBs can be found at low levels in the blood of almost everyone. These low levels are not currently known to cause any health problems. To reduce your intake of environmental contaminants which can accumulate in fish, we recommend that you use the Guide to Eating Ontario Sport Fish when choosing and cooking sport fish for eating.'	n/a
Women of reproductive age - ≥5 μg/L	'The level of PCBs in your blood was slightly higher than the acceptable level recommended by Health and Welfare Canada. The levels of the other environmental contaminants were within the range seen in the general population. We suggest you discuss these results with your doctor or the study's medical advisor, Dr. Donald Cole (at the number given above). Many environmental contaminants such as PCBs can be found at low levels in the blood of almost everyone. These low levels are not currently known to cause any health problems. To reduce your intake of environmental contaminants which can accumulate in fish, we recommend that you use the Guide to Eating Ontario Sport Fish when choosing and cooking sport fish for eating.'	≥5 μg/L: 'The PCB level of xxx is above the Health and Welfare 'tolerable level' of 5 μg/L for females of reproductive age, but below the tolerable level of 20 μg/L for males and post-menopausal women. The 5 μg/L tolerable level is based on a concern for the health of the developing fetus/infant. Mothers themselves are not likely at excess risk. Clinical history as to chloracne and liver problems may be worthwhile but further investigations are not warranted. Breast-feeding should continue to be encouraged. Discussion of sport fish consumption and occupational exposure (e.g. working with electrical transformers) may be helpful. Patients should be advised to use the Ministry of Environment Sport Fishing guidelines. Following the sport fish advisories will help to reduce blood PCB levels. Blood levels over the action level of 100 μg/L would signal the need to fully assess the health status of the individual and decrease exposure.'

PCBs (cont'd)		
Criteria	**Text used in Summary Sheet to Participants**	**Text used in Covering Letter to Physician**
Males and post-menopausal women - ≥20 μg/L	'The level of PCBs in your blood was slightly higher than the acceptable level recommended by Health and Welfare Canada. The levels of the other environmental contaminants were within the range seen in the general population. We suggest you discuss these results with your doctor or the study's medical advisor, Dr. Donald Cole (at the number given above). Many environmental contaminants such as PCBs can be found at low levels in the blood of almost everyone. These low levels are not currently known to cause any health problems. To reduce your intake of environmental contaminants which can accumulate in fish, we recommend that you use the Guide to Eating Ontario Sport Fish when choosing and cooking sport fish for eating.'	'The PCB level of xxx is above the Health and Welfare 'tolerable level' of 20 μg/L for males and post-menopausal women. Levels above 20 μg/L may be viewed with some concern. Clinical history as to chloracne and liver problems may be worthwhile but further investigations are not warranted. Discussion of sport fish consumption and occupational exposure (e.g. working with electrical transformers) may be helpful. Patients should be advised to use the Ministry of Environment Sport Fishing guidelines. Following the sport fish advisories will help to reduce blood PCB levels. Blood levels over the action level of 100 μg/L ppb would signal the need to fully assess the health status of the individual and decrease exposure.'

[1] Health Protection Branch, Foods Directorate memorandum, 1986.

Other organochlorines

No blood guidelines could be found for the other organochlorines except for WHO guidelines for total DDT (200 µg/L) and dieldrin + aldrin (100 µg/L)[1]. There were no exceedances of these values in our study.

For interpretation purposes, we explained to participants that the levels found in this study were similar or lower than levels found in other studies. This was included in the text provided above for PCBs. As well, on the data sheet, instead of providing guidelines for the organochlorines, estimated ranges of blood levels were provided based on a recent study by Williams et al (1988).[2] This study measured levels of organochlorines in adipose tissue of Great Lakes Basin residents; blood values were estimated assuming equivalent partitioning to blood lipids.

One exception was a person who had a mirex level of 1.3 µg/L. This value is at the higher end of levels seen in other studies so this was mentioned in the letters to this particular participant and physician (this person also had elevated PCB levels). The text used was as follows:

Text used in Summary Sheet to Participants	Text used in Covering Letter to Physician
'The level of PCBs in your blood was slightly higher than the acceptable level recommended by Health and Welfare Canada. The level of mirex in your blood sample was also slightly higher than the levels seen in the other participants and in other studies. The level of all other organochlorine contaminants, such as DDT, were within the range normally seen in the general population. We suggest you discuss these results with your doctor. You are also welcome to call the study's medical advisor, Dr. Donald Cole at 416-252-6471 (please call collect). Many environmental contaminants such as PCBs can be found at low levels in the blood of almost everyone living in an industrialized country. These low levels are not currently known to cause any health problems. Consumption of sport fish from Lake Ontario or the St. Lawrence River may have contributed to your levels of PCBs and mirex. Lake Ontario and the St. Lawrence River are contaminated with mirex due to discharges from two U.S. industrial sites into the Niagara and Oswego Rivers. Mirex is a pesticide and chemical additive that was manufactured at these sites in New York State until 1978. Mirex cannot be manufactured or used in Canada. To reduce your intake of environmental contaminants which can accumulate in fish, we recommend that you use the Guide to Eating Ontario Sport Fish when choosing and cooking sport fish for eating.'	'The PCB level of xxx is above the Health and Welfare 'tolerable level' of 20 µg/L for males and post-menopausal women. As well, the level of mirex in the blood sample was slightly higher than the levels seen in the other participants and in other studies. There is no 'tolerable' level' established for mirex in blood. The levels of the other environmental contaminants were within the range seen in the general population. The level of mirex does not indicate a health concern. PCB levels above 20 µg/L may be viewed with some concern. Clinical history as to chloracne and liver problems may be worthwhile but further investigations are not warranted. Discussion of sport fish consumption and occupational exposure (e.g. working with electrical transformers) may be helpful. Blood levels over the action level of 100 µg/L ppb would signal the need to fully assess the health status of the individual and decrease exposure. These levels indicate that the participant may have consumed considerable amounts of sport fish from Lake Ontario or the St. Lawrence River. Lake Ontario and the St. Lawrence River are contaminated with mirex due to discharges from two U.S. industrial sites into the Niagara and Oswego Rivers. Mirex is a pesticide and chemical additive that was manufactured at these sites in New York State until 1978. Mirex cannot be manufactured or used in Canada. Patients should be advised to use the Ministry of Environment Sport Fishing guidelines. Following the sport fish advisories will help to reduce intake of environmental contaminants and reduce blood levels.'

---

[1] (WHO Technical Report Series 677. Recommended health-based limits in occupational exposure to pesticides. World Health Organization. Geneva. 1982. Cited in: Human Toxicology of Pesticides by F.P. Kaloyanova and M.A. ElBatawi. CRC Press Inc. Florida. 1991, p. 91.)

[2] Williams et al. 1988. Organohalogen residues in human adipose autopsy samples from six Ontario municipalities. J. Assoc. Off. Anal. Chem. 7(2): 410-414.

Mercury (in whole blood and hair)

The following guidelines for total mercury were established by Health Canada[1]:

	Whole blood	Hair
normal acceptable range	≤20 ppb	≤6 ppm
'increasing risk'	20-100 ppb	6-30 ppm
'at risk'	>100 ppb	>30 ppm

All participants had blood and hair levels within the 'normal acceptable range' except for one person. This person had a hair level of 8.4ppm (in the 'increasing risk' category) but their blood value was well within the 'normal acceptable range'.

Mercury

Criteria	Text used in Summary Sheet to Participant	Text used in Covering Letter to Physician
Whole blood ≤20 ppb & Hair ≤ 6 ppm	'The levels of mercury, (cadmium) and (lead) found in your blood were similar to levels normally seen in the general population. The level of mercury found in your hair samples was also in the 'normal' range. All measurements were well below levels known to cause any health problems.'	n/a
One person with hair > 6ppm	'The mercury level in your hair was 8.4 parts per million (ppm). Health and Welfare Canada has suggested that levels between 6 and 30 ppm are 'of increasing risk'. Your value of 8.4 ppm is at the lower end of this range and the blood mercury value was well within the normal range. Therefore, we do not feel that this measurement is of great concern at this time. You may want to try to limit your mercury intake by using the Guide to Eating Ontario Sport Fish when choosing sport fish for eating. Aquatic animals such as muskrat may also be a source of mercury. You are welcome to discuss these results with the study's medical advisor, Dr. Donald Cole at 416-252-6471 (please call collect).	'The mercury level in the hair sample was 8.4 parts per million (ppm). Health and Welfare Canada has suggested that levels between 6 and 30 ppm are 'of increasing risk'. The value of 8.4 ppm is at the lower end of this range and the blood mercury value was well within the normal range. Therefore, we do not feel that this measurement is of great concern and no specific action is recommended. Patients should be advised to use the Ministry of Environment Sport Fishing guidelines when selecting sport fish for eating. Aquatic animals such as muskrat may also be a source of mercury.'

---

[1] Methylmercury in Canada. Vol II. 1984. Minister of National Health and Welfare. Ottawa, Canada. p. 21)

Lead (in whole blood μg/Dl)

The guideline of 25 μg/Dl developed by the Federal Provincial Advisory Committee on Lead was used in this study.

Lead		
Criteria	**Text used in Summary Sheet to Participant**	**Text used in Covering Letter to Physician**
<25 μg/Dl	** see text for normal mercury levels	n/a
≥25 μg/Dl	'The lead level in your blood was xxx ug/dL. This value is slightlv over the recommended acceptable blood level of 25 ug/dL. We suggest you discuss this result with your doctor at your next regular visit. You are also welcome to discuss this result with the study's medical advisor, Dr. Donald Cole. He can be reached at 416-252-6471 (please call collect).'	'The lead level just exceeds a guideline of 25 ug/dL established by the Federal Provincial Advisory Committee on Lead. It is common for people who have worked with products containing lead (gasoline, solder, paints) to have levels much higher than 25 ug/Dl. We note that the participant has worked as a mechanic. A repeat blood lead test is recommended to ensure that the lead level is not increasing (due to recent lead exposure). We further recommend that you discuss ways of reducing future exposure to lead in his daily work (or crafts). If symptoms are present or arise during a future stressful illness experience, specific investigations may document toxicity to the nervous, renal, reproductive, haematological or other systems."
clotted sample	'Unfortunately, we could not use the blood sample you gave us to measure the lead and cadmium levels. Due to technical problems the sample clotted too soon, so it was not possible to use the sample'.	'The measurements of blood lead and cadmium were not performed since the sample clotted prematurely'.

Cadmium (in whole blood, µg/L)

The Occupational Safety Health Administration has recommended a blood guideline for cadmium of 5 µg/L (for medical surveillance of American workers)[1]. The Ontario Ministry of Labour guideline is 11 µg/L[2]. In this study, recommendations are based on the 5 µg/L guideline.

Cadmium		
Criteria	**Text used in Summary Sheet to Participants**	**Text used in Covering Letter to Physician**
<5 µg/L		
≥5 µg/L	'The cadmium level in your blood was slightly higher than the recommended acceptable level. It is not clear if this level will be affecting your health but we suggest you discuss this result with your doctor or the study's medical advisor, Dr.Donald Cole.'	'The cadmium measure exceeds the guideline of 5 ug/l recently established by OSHA in the U.S. Some workplaces can be a source of cadmium exposure, however the most common source of cadmium exposure is from cigarette smoking. The range of blood cadmium levels in Canadian non-smokers has been estimated to be <2 ug/L; for smokers, <6 ug/L. Another possible source (but probably less important) is consumption of moose (or caribou) kidney or liver. We recommend conducting a more detailed exposure history and investigation of the patient's kidney function via urinalysis and urinary cadmium levels. If the exposure is occupational, this may be done through the Ministry of Labour laboratory.'
clotted sample	'Unfortunately, we could not use the blood sample you gave us to measure the lead and cadmium levels. Due to technical problems the sample clotted too soon, so it was not possible to use the sample'.	'The measurements of blood lead and cadmium were not performed since the sample clotted prematurely'.

---

[1] Occupational Safety and Health Administration, U.S. Department of Labour (1992): Occupational exposure to cadmium; final rules. Federal Register. 57:42102-42463

[2] Ontario Ministry and Labour (1991): Cadmium in blood and urine.

Liver activity measurements (in serum)

The following parameters were measured in serum by the Ottawa Civic Hospital. The reference ranges[1] and 'immediate notification' criteria[2] are also listed:

	Reference range	'Immediate notification criteria'
Albumin	35-48 g/L	<30 g/L
Alanine aminotransferase (ALT)	5-40 U/L	≥60 U/L or ≥50 U/L with elevated AST, GGT or ALP
Alkaline phosphatase (ALP) (U/L)	20-55 yr: 36-135 U/L 55-74 yr: 37-160 U/L	≥200 U/L
Aspartate aminotransferase (AST)	10-30 U/L	≥50 U/L or ≥40 with elevated ALT, GGT or ALP
Gamma glutamyl transferase (GGT)	males: 8-50 U/L females: 6-50 U/L	≥70 U/L

---

[1] Handbook of biochemical tests and reference values. 1992. Civic Hospital, Biochemistry Laboratory.
[2] Dr. S. Perkins, Ottawa Civic Hospital.

As described earlier, if any of the measurements exceeded the 'immediate notification' criteria, participants and physicians were notified as soon as the data was available. These results were repeated in the letters mailed in July 1993. The text used in the July letters was as follows:

Serum Liver Activity Measurements

Criteria	Text used in Summary Sheet to Participants	Text used in Covering Letter to Physician
All measurements within reference ranges	'The measurements of liver enzymes in your blood were within the normal range'.	n/a
One or more measurements outside of reference range but not exceeding 'immediate notification' criteria	'One or more of your liver enzyme measurements was slightly higher than normal. We do not feel this requires immediate attention but we suggest you discuss this result with your doctor at your next regular visit.'	'One or more of the liver enzyme measurements was slightly elevated. We recommend retesting if this finding is new and the clinical situation warrants.'
One or more measurements exceeded 'immediate notification' criteria (letters would have been sent earlier to participants and physicians)	'One or more of your liver enzyme measurements was higher than normal. We contacted you earlier this year about this result.'	'One or more of the liver enzyme measurements was elevated. This result was provided earlier this year.'

Urinary porphyrins

The following urinary porphyrins were measured in the grab sample by the Ottawa Civic Hospital. Urinary creatinine was also measured. If the creatinine value was outside of the normal reference range (given below), then all measured porphyrin values were adjusted for the creatinine level. The adjusted porphyrin values were then compared with the reference ranges for 24 hr. samples[1] (given below).

	Reference ranges[1] (24 hr. sample)
Uroporphyrin	0-42 nmol/d
Hepta, hexa, penta	<25 nmol/d
Coproporphyrin	0-245 nmol/d
Total porphyrin	≥350 nmol/d[2]
Creatinine	males: 12.5-16.0 ~~nmol/d~~ mmol/l females: 8.0-13.0 nmol/d mmol/l

---

[1] Reference ranges from: Handbook of biochemical tests and reference values. 1992. Civic Hospital, Biochemistry Laboratory.

[2] Reference range from Dr. Sherry Perkins, Ottawa Civic Hospital, pers. commun.

The following text was used in the July 1993 letters.

Urinary Porphyrins		
Criteria	**Text used in Summary Sheet to Participants**	**Text used in Covering Letter to Physician**
All measurements within reference ranges	The measurements of porphyrins (and D-glucaric acid) in your urine sample were also within the normal range.	n/a
One or more adjusted measurements outside of reference range	'One or more porphyrin levels in your urine sample was slightly higher than normal. We do not feel this requires immediate attention but we suggest you discuss this result with your doctor at your next regular visit.'	'One or more porphyrin levels in the urine sample was above the normal range, however, the levels are 10 fold less than levels seen in people with porphyria. We do not suggest retesting unless clinical signs develop.'

D-Glucaric Acid

D-glucaric acid in urine was measured by Dr. Raymond Poon, Environmental Health Directorate, Health Canada. No reference ranges exist for D-glucaric acid. The range found in this study was 1.41-655.53 μmoles/g creatinine. The median was 32.97 μmoles/g creatinine; the 95th percentile was 124.54 μmoles/g creatinine. It was decided that all people in the upper fifth percentile of the values found in this study would be considered 'elevated'. The following text was used in letters to participants and physicians:

Urinary d-glucaric acid

Criteria	Text used in Summary Sheet to Participants	Text used in Covering Letter to Physician
Value below 95th percentile	The measurements of (porphyrins and) D-glucaric acid in your urine sample (were) was within the normal range.	n/a
One or more adjusted measurements outside of reference range	'The level of d-glucaric acid in your urine sample was in the upper five percent of measurements seen in the study. The normal range of d-glucaric acid in the urine of the general population has not been determined. Various factors may affect d-glucaric acid levels including medications, age and perhaps environmental contaminants. Elevated d-glucaric acid is not an indication of liver damage however you may wish to discuss this result with your doctor at your next regular visit.'	'The level of d-glucaric acid in the urine sample was in the upper five percent of measurements seen in the study. D-glucaric acid is the metabolic endpoint of the glucuronic acid pathway for detoxification of toxic substances. Various factors may affect levels including medications, age and perhaps environmental contaminants. Elevated d-glucaric acid is not an indication of liver damage and no specific action is recommended.'

Thyroid hormones

The following thyroid hormones were measured in serum by the Ottawa Civic Hospital. Reference ranges are also provided. As with the lipids and liver activity measurements, participants (and physicians) were contacted as soon as the data was available, if one of the thyroid measurements exceeded the 'immediate notification' criteria. This 'immediate notification' criteria was developed in discussion with Dr. S. Perkins, Ottawa Civic Hospital.

	Reference Range[1]	'Immediate notification' criteria[2]
Free thyroxine (FT_4)	11.0-23.0 pmol/L	$FT_4 \geq 23.0$ pmol/L and TSH ≤ 0.1 mU/L
Thyrotropin (TSH) (mU/L)	0.4-5.0 mU/L	or
		$FT_4 \leq 11.0$ pmol/L and TSH ≥ 7.0 U/L
Triiodothyronine (T_3)	0.80-2.70 nmol/L	none

[1] Handbook of biochemical tests and reference values. 1992. Civic Hospital, Biochemistry Laboratory.
[2] Dr. Sherry Perkins, Ottawa Civic Hospital, pers. commun.

Thyroid hormones		
Criteria	**Text used in Summary Sheet to Participants**	**Text used in Covering Letter to Physician**
All measurements within reference range	'The measurements of thyroid hormones in your blood were within the normal range'.	n/a
One or more measurements outside of reference range but not meeting 'immediate notification' criteria	'One or more of your thyroid hormone measurements was slightly outside the normal range. We do not feel this requires immediate attention but we suggest you discuss this result with your doctor at your next regular visit.'	'One or more of the thyroid hormones measurements was slightly outside of the reference range. We recommend retesting if this finding is new and the clinical situation warrants.'
One or more measurements exceeded 'immediate notification' criteria (participant and physician already contacted)	'One or more of your thyroid hormone measurements was outside of the normal range. We contacted you earlier this year about this result.'	'One or more of the thyroid hormones was outside of the reference range. This result was provided earlier this year.'

Appendix 8
Examples of letters with preliminary clinical chemistry results
(sent Jan/Feb 1993)

Letter sent to participant with initial clinical chemistry results

Great Lakes Health Effects Program
Room 203
Environmental Health Centre
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

January 15, 1992

[Participant name]
[Participant address]

Dear [Participant name],

We want to thank you very much for your recent participation in the Great Lakes Basin Anglers Study. We have started to get laboratory results for some of the samples you provided to us. Our medical advisor felt that the results from some of the blood tests should be followed up by you and your family physician.

As you requested on the consent form, we have sent those results which are available now, to your family physician, [Physician name]. Please contact him/her to discuss the results and any further follow-up that may be required.

If you have any further questions after meeting with your physician, you can call the project's medical advisor, Dr. Donald Cole, at the LAMP Occupational Health Program, (416)-252-6471. Please call collect.

We will be in touch with you as other results become available over the coming months. Thank you again for your participation in the Great Lakes Basin Anglers Study.

Sincerely,

Jill Kearney
Project coordinator
Great Lakes Basin Anglers Study
Health and Welfare Canada

Dr. Donald Cole
Project medical advisor
Great Lakes Basin Anglers Study
LAMP Occupational Health Program

Letter sent to physician with initial clinical chemistry results

Great Lakes Health Effects Program
Room 203
Environmental Health Centre
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

January 12, 1993

Dr. [Physician name]
[address]

Dear Dr. [Physician name]

Re: [Participant name]
[Participant address]

[Participant phone no.]
[Participant birthdate]

As you may be aware, the person listed above recently participated in the Great Lakes Basin Anglers Study. This is a research project being carried out by Health and Welfare Canada, examining the relationship between consumption of contaminated fish from the Great Lakes Basin and human absorption of environmental contaminants such as PCBs and mercury. Blood, hair and urine samples are being analyzed for a variety of environmental contaminants as well as clinical chemistry parameters and selected biomarkers (eg. sister chromatid exchanges).

On the informed consent form the participant indicated that they would like all test results to be forwarded to you. Initial clinical chemistry results, done by the Ottawa Civic Hospital are now available. Some of the results for [Participant name] are sufficiently out of normal range that the clinical biochemist felt these results should be brought to your attention for follow-up, as necessary. The test results are listed on the attached information sheet, along with the reference values used by the laboratory. It should be noted that the participants were not requested to fast prior to the blood sample being taken. We have informed the participant that the test results have been sent to you, and have suggested that they discuss them with you.

We do not have environmental contaminant data or other biomarker data at this time to interpret these results in light of fish consumption. This data will be available in the coming months and will be sent to you at that time.

If you would like to discuss these results or any other medical aspects of the study, please contact the study's medical advisor, Dr. Donald Cole. You may call him collect at the LAMP Occupational Health Program, 185 Fifth St., Etobicoke Ont., 416-252-6471.

.../2

Thank you for your assistance. If you have any other questions about the study, please contact Jill Kearney, project co-ordinator, at 613-957-8487.

Sincerely,

Jill Kearney	Dr. Donald Cole
Project coordinator	Project medical advisor
Great Lakes Basin Anglers Study	Great Lakes Basin Anglers Study
Health and Welfare Canada	LAMP Occupational Health Program

Appendix 9
Examples of material to participants and physicians for reporting of all results
(sent July 1993)

Covering letter to Participants

Great Lakes Health Effects Program
Health and Welfare Canada
1100 Block, Main Building
Tunney's Pasture
Ottawa, Ontario
K1A 0L2

July 7, 1993

(Participant name)
(Participant address)

Dear (Participant name),

We want to extend our thanks to you again for your participation in the Great Lakes Basin Anglers Study. We are now able to give you a summary of your laboratory results from your blood, hair and urine samples. As you requested, a complete list of the laboratory results has been sent to your doctor, Dr. (Physicians name).

The summary of your laboratory results is on the next page. Please remember that the results of these tests are affected by many factors other than fish consumption, such as smoking, medications, occupation or your general health. If you have any concerns or questions, we suggest you talk to your doctor or you are welcome to call our medical advisor, Dr. Donald Cole. He may be reached at 416-252-6471 (please call collect).

In the upcoming months, we will be looking at the relationship between these measurements and sport fish consumption and other lifestyle factors. A summary of our findings will be sent to you as soon as they are available, probably next winter. In the meantime, if you have any questions about the study in general, please call Jill Kearney at 613-957-8487 or write at the above address.

Have a great summer! For those who fish, we hope you have a great fishing season. The new version of the Guide to Eating Ontario Sport fish is now out. Look for it at local beer and liquor stores. If you are interested in learning more about environmental pollutants and fish, we have included a brochure called "Having Your Catch and Eating It Too!".

Thank you again for your support in this study.

Sincerely,

Jill Kearney
Study coordinator

Dr. Donald Cole
Medical advisor

Great Lakes Basin Anglers Study
Summary of participant laboratory results
June 1993

Participant: (Participant name)
(Participant address)

Sample date:

Environmental Contaminants

Metals

The levels of mercury, lead and cadmium found in your blood and the level of mercury found in your hair were similar to levels normally seen in the general population. All measurements were well below levels known to cause any health problems.

Organochlorine contaminants (eg. PCBs, DDT etc.)

The levels of PCBs and the other environmental contaminants such as DDT, in your blood, were in the range normally found in the Canadian general population. Many environmental contaminants such as PCBs can be found at low levels in the blood of almost everyone. These low levels are not currently known to cause any health problems. To reduce your intake of environmental contaminants which can accumulate in fish, we recommend that you use the Guide to Eating Ontario Sport Fish when choosing and cooking sport fish for eating.

Clinical chemistry

Cholesterol and triglyceride: Your cholesterol level and/or triglyceride level was slightly higher than the recommended level. We do not feel this requires immediate attention but we suggest you discuss this result with your doctor at your next regular visit.

Measurements related to liver activity: One or more of your liver enzyme measurements was slightly outside of the normal range. We do not feel this requires immediate attention but we suggest you discuss this result with your doctor at your next regular visit.

The measurements of porphyrins and D-glucaric acid in your urine sample were within the normal range.

Thyroid hormones: The measurements of thyroid hormones in your blood were within the normal range.

Covering letter to Physicians

Great Lakes Health Effects
Program
1100 Block
Main Building
Tunney's Pasture
Ottawa, Ontario
K1A 0K9

July 7, 1993

Dr. (Physician name)
(Physician address)

Dear Dr.(Physician name)

Re: (Participant name)
(Participant address)

As you may be aware, (Participant name) recently participated in the Great Lakes Basin Anglers Study. This is a research project being carried out by Health and Welfare Canada, examining the relationship between reported consumption of fish from the Great Lakes Basin and human levels of environmental contaminants such as PCBs and mercury. Blood, hair and urine samples from the participants have been analyzed for a variety of environmental contaminants as well as clinical chemistry parameters and selected biomarkers (urinary porphyrins, D-glucaric acid).

On the consent form the participant indicated that they would like all test results to be forwarded to you. These test results are listed on the attached information sheet, along with the reference values or guidelines used for flagging results which may require follow-up.

We have commented below on those results which we would like to bring to your attention for follow-up, as necessary. We have included a brief summary of our interpretation and recommendations in light of these results:

> The cholesterol and/or triglyceride value was slightly elevated, but this result was from a non-fasted sample. As well, one or more of the liver enzyme measurements was slightly outside the normal range. We recommend retesting if these findings are new and the clinical situation warrants.
>
> All other results were within the 'normal' ranges.

If you would like to discuss these results or any other medical aspects of the study, please contact the study's medical advisor, Dr. Donald Cole. You may call him collect at the LAMP Occupational Health Program, 185 Fifth St., Etobicoke Ont., 416-252-6471.

You or your patients may be interested in the enclosed brochure entitled "Having Your Catch and Eating It Too" which provides more information about environmental contaminants and sport fishing.

Sincerely,

Jill Kearney
Study coordinator

Dr. Donald Cole
Medical advisor

Great Lakes Basin Anglers Study
Laboratory Results
June 1993

Participant: Name
Address

Parameter	Measured value	Comparison levels	Description
Metals		Tolerable levels	
Total mercury (in whole blood)	3 ppb	20 ppb (Health and Welfare Canada)	Mercury is an environmental contaminant which, in the organic form called methylmercury, can accumulate in fish living in lakes with elevated mercury levels. At high levels, methylmercury affects the nervous system. Mercury can be measured in blood and hair samples.
Total mercury (in hair)	N.D.	6 ppm (Health and Welfare Canada)	
Cadmium (in whole blood)	N.D.	5 μg/L (U.S. Occupational Safety and Health Administration)	Cigarettes are probably the most important source of general cadmium exposure. Cadmium is also known to accumulate in the kidney and liver of some mammals such as moose and caribou. At high levels cadmium can affect the kidney.
Lead (in whole blood)	4.8 μg/dL	25 μg/dL (Federal Provincial Advisory Committee on Lead)	Lead is an important workplace and environmental contaminant. Consumption of sport fish is not a major route of exposure. Consumption of lead shot in wildlife could elevate blood lead levels. At high levels lead can affect the nervous system.
Organochlorines (in blood plasma)			
PCBs	9.8 μg/L	5 μg/L for women of reproductive age 20 μg/L for post-menopausal women and men (Health and Welfare Canada)	Many of the organochlorine environmental contaminants can be found at low levels in almost everyone living in an industrialized country. It has only been in the last 5 years or so that the technology has been available to make it practical to measure these contaminants at such low levels in human blood samples. Consequently there is little data on blood levels to use for comparison purposes.
DDT	0.19 μg/L	200 μg/L (DDT+DDE) (World Health Organization)	
DDE	1.7 μg/L		
Dieldrin	0.14 μg/L	100 μg/L (Dieldrin+Aldrin) (World Health Organization)	
		Estimated ranges in general population	
Hexachloro-benzene (HCB)	0.28 μg/L	0.15-3.13 μg/L	The estimated blood level ranges provided in the table have been calculated from adipose (fat) tissue levels measured in samples taken during autopsies of 142 people in 6 Great Lakes municipalities in 1984. These estimates assume that the contaminants are at the same concentration on a lipid basis in the adipose and the blood. The adipose levels were reported by D. T. Williams et. al. (1988) in "Organohalogen residues in human adipose autopsy samples from six Ontario municipalities" (J. Assoc. Off. Anal. Chem. 7(2): 410-414.)
ß-hexachloro-cyclohexane (HCH)	0.20 μg/L	0.12-4.44 μg/L	
α-chlordane	N.D.	not detected	
δ-chlordane	N.D.	not detected	
oxychlordane	0.25 μg/L	0.07-1.99 μg/L	
transnonachlor	0.61 μg/L	0.12-3.91 μg/L	
heptachlor epoxide	N.D.	0.02-1.26 μg/L	
mirex	0.07 μg/L	<0.014-0.82 μg/L	

*** An asterisk denotes a value above the guideline or outside the estimated range in the general population.**
"N.D." means the parameter was not detectable (below detection limit).

... 2

Great Lakes Basin Anglers Study
Laboratory Results (continued)

Participant: (Participant name)

Parameter	Measured value		Comparison levels	Description
			Reference ranges	
Lipids (fats) (serum)				
Total cholesterol	6.0 mmol/L	*	≤5.2 mmol/L	These were measured to estimate the total lipids in the blood. Note that measurements were taken on a non-fasted sample but reference ranges are for fasted samples. Triglyceride values above 4 mmol/L have been marked with an asterisk.
Triglycerides	1.87 mmol/L		0.55-1.70 mmol/L	
Free cholesterol	1.49 mmol/L		not available	
Phospholipids	2.55 mmol/L		not available (Ottawa Civic Hospital)	
Liver related measurements in serum				
Albumin	43 g/L		35-48 g/L	At higher levels, some environmental contaminants have been seen to increase liver marker enzymes in blood. As well, some liver diseases may affect the way environmental contaminants are handled by the body.
Alanine aminotransferase (ALT)	44 U/L	*	5-40 U/L	
Alkaline phosphatase (ALP)	73 U/L		37-160 U/L	
Aspartate aminotransferase (AST)	31 U/L	*	10-30 U/L	
Gamma glutamyl transferase (GGT)	39 U/L		8-50 U/L (Ottawa Civic Hospital)	
Urinary porphyrins				
Uroporphyrin	27.4 nmol/d		0-42 nmol/d	These values for nmols per day have been estimated from the 'first morning void' samples, by adjusting for urine creatinine level. At higher levels, some environmental contaminants have been related to porphyria.
Heptaporphyrin	4.3 nmol/d		<25 nmol/d	
Hexaporphyrin	N.D.		<25 nmol/d	
Pentaporphyrin	2.0 nmol/d		<25 nmol/d	
Coproporphyrin	208.6 nmol/d		0-245 nmol/d (Ottawa Civic Hospital)	
Urinary D-glucaric acid	21.41 µmoles/g creatinine		Range for this study: 1.41-655.53 µmoles/g creatinine Median for this study: 32.97 µmoles/g creatinine	There is no established reference range for D-glucaric acid in urine. Various factors may affect levels including medications, age and perhaps environmental contaminants. Elevated D-glucaric acid is not an indication of liver damage.
Thyroid hormones (serum)				
Free thyroxine (FT4)	19.1 pmol/L		11.0-23.0 pmol/L	At higher levels, some environmental contaminants affect thyroid function.
Thyrotropin (TSH)	2.3 mU/L		0.4-5.0 mU/L	
Triiodothyronine (T3)	1.6 nmol/L		0.80-2.70 nmol/L (Ottawa Civic Hospital)	

*An asterisk denotes a value outside the reference range (or for D-glucaric acid, in the upper 5th percentile).
"N.D." means the parameter was not detectable (below detection limit).